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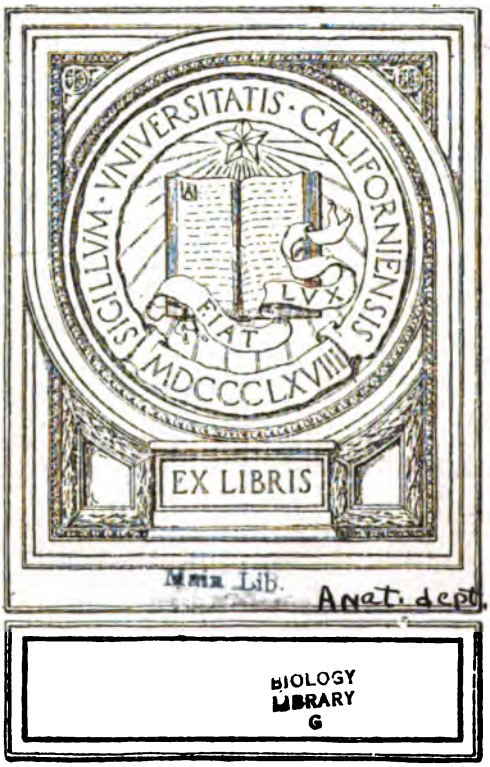


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INCLUDING

## THE MICROSCOPIC TECHNIC

BY

DR. PHILIPP STÖHR

PROFESSOR OF ANATOMY AT THE UNIVERSITY OF WÜRZBURG

University of California

*SECOND AMERICAN FROM EIGHTH GERMAN EDITION*

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chapters. Furthermore, we have enlarged the chapter on the Uterus, in order to give detailed consideration to the various functional conditions of the organ, and added to the book an entirely new chapter on the Placenta. Eight new illustrations (Figs. 229, 230, 232, 233, 234, 236, 237, 238) were necessary for these additions.

The editor is under great obligation to the translator, Dr. Billstein, for her successful efforts in reproducing the conciseness and clearness of the German original. Further, he desires to express his gratitude to Prof. Philipp Stöhr for placing at his disposal the original electrotypes, and to Drs. Böhm and von Davidoff for the illustration of the virginal uterus (Fig. 229) from their "Lehrbuch der Histologie." He also feels deeply indebted to Prof. Charles S. Minot for kind assistance, for valuable criticism, and for permission to use two illustrations (Figs. 231 and 234) from his text-book of "Human Embryology"; and, finally, to Messrs. P. Blakiston, Son & Co., Philadelphia, for the very satisfactory reproduction of the new drawings, and for their many courtesies during the preparation of the American edition.

ALFRED SCHAPER.

HARVARD MEDICAL SCHOOL,
BOSTON, *June, 1896.*

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PART I.

# GENERAL TECHNIC.

## I. THE LABORATORY APPOINTMENTS.

### 1. INSTRUMENTS.

**The Microscope.**—From my own experience I can recommend the microscopes made in the optical works of Leitz in Wetzlar, Seibert in Wetzlar, and Zeiss in Jena, having repeatedly tested their excellent workmanship.*

It is not advisable for the beginner to purchase a microscope without first submitting it to an expert for examination. In order to preserve the microscope in good working condition it is necessary to protect it from dust; when in frequent use it is best to keep it under a bell-glass, in a place not exposed to sunlight. The tarnish which forms on the tube should be rubbed off with a dry piece of soft filter-paper.

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* Students of the first semester are advised to refrain from the purchase of high-power oculars and immersion-systems. These should be bought shortly before entering upon bacteriological work.

The following outfits are recommended:

*Leitz.*—Catalogue No. 36, 1895. Microscope No. 4 *b*. Price, 370 *M.* = $92.00. Without homogeneous immersion and ocular IV, 265 *M.*

*Seibert.*—Catalogue No. 25, 1895. Microscope 3 *c*. Price, 449 *M.* = $112.00. Without homogeneous immersion, objective 3, and ocular 0, 283.50 *M.* = $71.00.

*Zeiss.*—Catalogue No. 30, 1895. Combination (p. 116) 7 *b*. Price, 602 *M.* = $150.00. Without homogeneous immersion, 442 *M.* = $110.00; or 8 *b*. Price, 559 *M.* = $140.00. Without homogeneous immersion, 399 *M.* = $100.00.

The majority of the work for this book was carried out with a Leitz microscope.

*Editor's remark:* Of American microscopes those made by the Bausch & Lomb Optical Co., Rochester, N. Y., and New York City, are recommended.

For histological work the following outfit is suitable:

Stand BB.—Oculars, 1-inch and 3-inch. Objectives, ⅔-inch and ⅛-inch. Catalogue 1895. Price, $62.50. For cytological and bacteriological work a $\frac{1}{12}$-inch oil-immersion objective (price, $44) and an Abbé condenser and iris-diaphragm should be added. For convenience a double or triple revolver for the objectives is desirable.

Smirches on the lenses and on the mirrors should be removed with soft eather, and if this does not answer the purpose—as, for example, when a lens is smeared with Canada-balsam or damar-varnish—a small piece of fine linen moistened with a drop of pure alcohol should be used. In the latter procedure great care must be exercised lest the alcohol penetrate the setting of the lenses and dissolve the balsam with which they are cemented together. Therefore the balsam should be *quickly* rubbed off with the moistened linen and the lens carefully dried. The screws of the microscope should be cleaned with benzin. The lenses of the objective must not be unscrewed.

A good *razor*, flat on one side. It should always be kept sharp, and before each use should be drawn without pressure over the *strop*. The honing of it should be left to the instrument-maker. The razor should be used only in the preparation of microscopic sections.

A fine *whetstone*.

A pair of fine, straight *scissors*.

A pair of easily-closing fine *forceps*, with smooth or only slightly notched points.

Four *dissecting needles* with wooden holders: two are to be heated, then slightly bent, heated again and thrust into solid paraffin, by which they are again hardened. The other two must be kept clean and sharply pointed; for delicate dissections the needles must be pointed and polished first on the whetstone and then on the strop.

A flexible *section-lifter*, for the transfer of sections from fluids to the slide, is very useful but not absolutely necessary. A scalpel having a broad blade can be used instead.

*Pins*, *quills*, *cork disks*, a fine *sable brush*.

A *crayon*, for writing on glass. If the glass be oily, it should first be cleansed with a little alcohol.

*Slides*, of clear glass and not more than 1 to 1.5 mm. in thickness.

*Cover-glasses*. Those measuring 15 to 18 mm. in diameter are generally large enough; the thickness may vary from 0.1 to 0.2 mm.

*Small wide-mouthed bottles*. One dozen, capacity 30 c.c. and over, with cork stoppers.

Several glass *preparation jars* (preserve jars), with tightly-fitting covers. Height, 8 to 12 cm.; diameter, 6 to 10 cm.

A *cylindrical graduate*, capacity 100 to 150 c.c.

A glass *funnel*, upper diameter 8 to 10 cm.

A *pipette*. Small pipettes may be prepared by heating in a gas-flame a glass tube 1 cm. thick and 10 cm. long, pulling one end to a point and placing on the other a small rubber bulb.

A dozen *watch-glasses* of 5 cm. diameter.

A dozen *test-tubes* 10 cm. long and 12 mm. wide.

*Glass rods* 3 mm. thick, 15 cm. long, some drawn to a point at the end.

Old bottles that have been thoroughly cleansed will answer as receptacles for reagents. In most cases the bottles can be cleansed with water, but sometimes it is necessary to rinse them with crude hydrochloric acid or with potash lye, then with ordinary water, then with distilled water, and finally with alcohol.

*Glass dishes* (Stender dishes), 6 to 8 cm. in diameter, with ground covers, are not absolutely necessary, but very useful.* In many cases they may be replaced by saucers, food dishes for birds, etc.

A few sheets of thin, white *filter-paper*, large and small gummed labels, soft pieces of linen (old handkerchiefs), a towel, a large and a small bottle-brush.

A large earthen jar for refuse.

## 2. REAGENTS.†

**General Rules.**—Large quantities of reagents should not be kept on hand, because many decompose in a comparatively short time. Certain reagents (see below) should be procured or prepared shortly before they are to be used. Each bottle should be provided with a large label on which its contents are designated; it is advisable to write on the label not only the formula of the reagent, but also the mode of its application. All the bottles must be tightly closed with cork or good glass stoppers. The fluid should not reach to the lower surface of the cork.

1. *Distilled water*, 3 to 6 liters.

2. *Normal salt solution*, 0.75 per cent. (sodium chlorid, 1.5 gm.; distilled water, 200 c.c.).

The cork must be provided with a glass rod reaching to the bottom of the bottle. This solution spoils easily and must be frequently prepared afresh.

3. *Alcohol.* (*a*) *Ninety-five per cent. Alcohol.*—About 500 c.c. should

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* Most of the glassware, including slides and cover-glasses, here enumerated may be obtained of W. P. Stender, Leipzig; or, in the United States, of the Bausch & Lomb Optical Co., New York.

† The reagents must be obtained from a reputable dealer. Excellent dyes and reagents may be had of Dr. Grübler, chemical and physiological laboratory, Leipzig, Bayer'sche Strasse 63. In the United States Grübler's stains and reagents are sold by Eimer & Amend, New York, and others.

be kept on hand. The alcohol of commerce is 95 per cent., and in the majority of cases is entirely satisfactory for microscopic purposes. If it is desired to obtain alcohol free from water (absolute alcohol), drop into the bottle a few pieces of copper sulphate heated to white heat (15 gm. to 100 c.c. of alcohol). When these become blue they must be replaced by new pieces or be reheated. Fresh quicklime serves the same purpose, but acts more slowly.*

(*b*) *Ninety per cent. Alcohol.*—Prepare 500 c.c. by diluting 475 c.c. of 95 per cent. alcohol with 25 c.c. of distilled water.

(*c*) *Seventy per cent. Alcohol.*—Prepare 500 c.c. by mixing 370 c.c. of 95 per cent. alcohol with 130 c.c. of distilled water.

(*d*) *Fifty per cent. Alcohol.*—Prepare 500 c.c. by mixing 265 c.c. of 95 per cent. alcohol with 235 c.c. of distilled water.

(*e*) *Thirty-three per cent. Alcohol* (Ranvier's one-third alcohol).—This is prepared by mixing 40 c.c. of 95 per cent. alcohol with 60 c.c. of distilled water.

4. *Acetic Acid*, 59 c.c.—The official is 30 per cent.

5. *Glacial Acetic Acid.*—This should be procured shortly before it is required. The commercial acid is 96 per cent.

6. *Nitric Acid.*—A bottle holding 100 c.c. of concentrated nitric acid of 1.18 sp. gr. (containing 32 per cent. of acid hydroxid) should be kept in stock.

7. *Hydrochloric acid*, pure, 50 c.c.

8. *Chromic Acid.*—A 10 per cent. stock solution should be prepared by dissolving 10 gm. of fresh crystalline chromic acid in 90 c.c. of distilled water. From this can be made:

(*a*) A 0.1 per cent. chromic-acid solution (10 c.c. of stock solution to 990 c.c. of distilled water), and—

(*b*) A 0.5 per cent. chromic-acid solution (50 c.c. of stock solution to 950 c.c. of distilled water).

9. *Potassium Bichromate.*—This should be kept on hand in two solutions:

---

* For the preparation of mixtures containing a smaller percentage of alcohol this equation will serve:

$$100 : 95 = x : \%$$

*e. g.*, 90%, $100 : 95 = x : 90$

$$95x = 90.\ 100$$

$$x = \frac{9000}{95} = 94.7 \text{ or } 95.$$

Therefore, to obtain 100 c.c. of 90 per cent. alcohol, 95 c.c. of 95 per cent. alcohol must be mixed with 5 c.c. of distilled water. For our purposes the errors of this ratio are too insignificant for consideration.

(*a*) 25 gm. to 1000 c.c. of distilled water.

(*b*) 35 gm. to 1000 c.c. of distilled water (for the Golgi mixture, No. 12).

At room temperature it dissolves in from three to six days. Therefore make the solutions with warm water or place the bottles near the stove.

10. *Müller's Fluid.*—Dissolve 30 gm. of sodium sulphate and 60 gm. of pulverized potassium bichromate in 3000 c.c. of distilled water. The solution can be made with the aid of heat, like No. 9.

11. *Zenker's Fluid.*—Dissolve 25 gm. of potassium bichromate, 10 gm. of sodium sulphate, and 50 gm. of mercuric chlorid in 1000 c.c. of warm distilled water. Before using add 1 c.c. of glacial acetic acid to each 20 c.c. of the mixture.

12. *Golgi's Mixture* (osmio-bichromate mixture).—This is prepared by pouring together 54 c.c. of the 3.5 per cent. solution of potassium bichromate (9 *b*) and 6 c.c. of the 2 per cent. osmic-acid solution (No. 19). It should be prepared shortly before it is to be used.

For the "fixation" of the Golgi preparations the following solutions are required:

13. *Stronger Hydrochinone Developer.*—This consists of 5 gm. of hydrochinone, 40 gm. of sodium sulphite, 75 gm. of potassium carbonate, and 250 gm. of distilled water. From this prepare a dilution by adding 20 c.c. of the mixture to 230 c.c. of distilled water. In a well-closed bottle in a dark place it keeps for weeks. The yellowish coloration which appears in time does not depreciate it.

14. *Sodium Hyposulphite* (10 gm. in 50 c.c. of distilled water).—It dissolves quickly without the aid of heat.

15. *Cox-Golgi Mixture.*—This is prepared by pouring together 40 c.c. of a 5 per cent. solution of potassium bichromate, 40 c.c. of a 5 per cent. solution of corrosive sublimate, 32 c.c. of a 5 per cent. solution of potassium chromate, and 88 c.c. of distilled water. This mixture may be kept in stock.

16. *Picric Acid.*—Keep on hand 50 gm. of the crystals and 500 c.c. of a saturated aqueous solution, in which undissolved crystals in a stratum 2 to 3 mm. deep must always lie on the bottom of the bottle. It dissolves readily.

17. *Picrosulphuric Acid* (Kleinenberg's solution).—This is prepared by adding 4 c.c. of pure sulphuric acid to 200 c.c. of a saturated aqueous solution of picric acid; a copious precipitate occurs. In about one hour filter the mixture and dilute the filtrate with 600 c.c. of distilled water. The residue on the filter is to be thrown into the refuse jar.

18. *Chromic-acetic Acid.*—To 50 c.c. of the 0.5 per cent. chromic acid solution (8 *b*) add 50 c.c. of distilled water and 3 to 5 drops of glacial acetic acid.

19. *Osmic Acid.*—This may be obtained from the dealer—50 c.c. of a 2 per cent. solution—shortly before it is needed. It is very expensive. It should be kept in the dark or in a dark glass bottle, and if well-stoppered can be preserved many months.

20. *Chromic-acetic-osmic Acid* (Flemming's mixture).—Prepare a 1 per cent. chromic-acid solution (5 c.c. of the 10 per cent. solution [No. 8] to 45 c.c. of distilled water) and add 12 c.c. of 2 per cent. osmic acid and 3 c.c. of glacial acetic acid. This mixture is not injured by light and can be kept in stock.*

21. *Platinum Chlorid.*—Prepare a 10 per cent. stock solution, 2 gm. dissolved in 20 c.c. of distilled water.

22. *Platinum-acetic-osmic Acid Mixture.*—Pour into 60 c.c. of a 1 per cent. solution of platinum chlorid (6 c.c. of stock solution and 54 c.c. of distilled water) 8 c.c. of 2 per cent. osmic acid solution and 4 c.c. of glacial acetic acid.

23. *Silver Nitrate.*—A 1 per cent. solution (1 gm. of silver nitrate in 100 c.c. of distilled water) should be procured a short time before it is to be used. In a dark place or in a dark bottle it can be preserved for a long time.

24. *Gold Chlorid.*—A solution of 1 gm. of gold chlorid in 100 c.c. of distilled water should be procured shortly before it is to be used. It must be kept in the dark or in a dark bottle. For gold-chlorid staining it is necessary to have the following:

25. *Formic acid*, 50 c.c.

26. *Concentrated potash lye* (35 per cent.), 30 c.c. The bottle must have a rubber stopper that is pierced by a glass rod. It should be procured from the druggist.

27. *Glycerol.*—One hundred c.c. of pure glycerol are to be kept in stock; also a solution of 5 c.c. of pure glycerol in 25 c.c. of distilled water. The growth of fungi, which soon takes place in this mixture, may be prevented by the addition of a small piece of camphor or thymol. The cork of the bottle should be provided with a glass rod.

28. *Bergamot oil* (green), 20 c.c. The bottle should have a cork pierced by a glass rod. The much-used cheaper clove oil scents the whole laboratory and its occupants.

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* Tissues fixed in old Flemming's fluid often stain badly, because the acetic acid has evaporated; 5 to 20 drops of acetic acid newly added to the solution removes this defect.

(*a*) *Xylol.*—This is to be used in special cases instead of bergamot oil. Xylol clears more strongly, and, on account of its sensitiveness in preparations incompletely dehydrated, is not recommended to beginners.

29. *Damar-varnish* (of Dr. Fr. Schönfeld & Co. in Düsseldorf) may be purchased in small bottles containing about 50 c.c. from dealers in artists' materials. If it is too thick it may be diluted with pure turpentine. It has the proper consistence when the drops from an immersed glass rod fall without spinning long threads. Damar is preferable to Canada-balsam (diluted with chloroform), which clears too vigorously and renders tissues too transparent, but has the disadvantage of drying more slowly than balsam. The cork of the bottle should be provided with a glass rod.*

(*a*) *Xylol-balsam*, solution of Canada-balsam in xylol, a substitute for damar-varnish.

30. *Cover-glass Cement.*—Dilute Venetian turpentine with enough ether to make an easily-flowing liquid; then filtered warm (in a heated funnel) and the filtrate inspissated on a sand-bath. The proper consistency is attained when a drop transferred with a glass rod to a slide hardens at once and becomes so firm that it cannot be indented with the finger-nail. Because of the danger of fire, it is better to have the cement prepared by the druggist.†

31. *Hansen's Hematoxylin.*—(*a*) Dissolve 1 gm. of crystallized hematoxylin in 10 c.c. of absolute alcohol and preserve it in a stoppered bottle. (*b*) Dissolve 20 gm. of potassium alum in distilled water, with the aid of heat, and when cold filter. (*c*) Dissolve 1 gm. of potassium permanganate in 16 c.c. of distilled water, at room-temperature. On the next day pour solutions *a* and *b* into a porcelain capsule, add 3 c.c. of solution *c*, and, with constant stirring, heat the mixture to boiling and boil about one minute. Cool quickly by floating the porcelain capsule in cold water. When cold the mixture should be filtered; it is then ready to use. Cloudiness, or the development of fungi in the mixture, does not depreciate its effectiveness in the slightest degree. It is to be kept on hand.

32. *Delafield's Hematoxylin.*—(*a*) Dissolve 1 gm. of crystallized hematoxylin in 6 c.c. of absolute alcohol. (*b*) Dissolve 15 gm. of ammonia alum in 100 c.c. of distilled water, with the aid of heat, and when cold filter. Pour the two solutions together and let the mixture

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* *Editor's remark:* Instead of this commercial damar-varnish I recommend a solution of pure gum damar in xylol, which has the advantage of drying more quickly.

† *Editor's remark:* In the United States an excellent fluid cover-glass cement is prepared by J. D. King, Cottage City, Mass.

stand three days in a wide-open vessel exposed to the light; then filter and mix with 25 c.c. of pure glycerol and 25 c.c. of methyl-alcohol. After three days filter the mixture. It does not deteriorate with age and should be kept in stock.

33. *Weigert's hematoxylin*, for the demonstration of the medullated nerve-fibers of the brain and the spinal cord. Heat 1 gm. of crystallized hematoxylin in 10 c.c. of absolute alcohol plus 90 c.c. of distilled water, and when cold filter. It should be prepared shortly before it is to be used. The application of this stain demands the aid of the following three fluids:

34. *Saturated Solution of Lithium Carbonate.*—Dissolve 3 or 4 gm. of lithium carbonate in 100 c.c. of distilled water. This should be prepared the day before using.

35. *Solution of Potassium Permanganate* (0.25 per cent.).—Dissolve 0.5 gm. of potassium permanganate in 200 c.c. of distilled water. This is to be kept on hand.

36. *Acid Mixture* (Pal's mixture).—Dissolve 1 gm. of pure oxalic acid and 1 gm. of potassium sulphite ($K_2SO_3$) in 200 c.c. of distilled water. This mixture should be prepared one day before using and kept in a well-stoppered bottle.

37. *Neutral Carmine-solution.*—Dissolve 1 gm. of the best carmine in 50 c.c. of cold distilled water to which 5 c.c. of a solution of ammonia, sp. gr. 0.960 (liquor ammonii caustici) have been added. The deep, cherry-red fluid should stand in an open vessel until it has no odor of ammonia (about three days) and then be filtered. It is to be kept in stock. The odor of this solution immediately becomes very disagreeable, but this does not depreciate its staining power.

38. *Picrocarmine.*—Pour 5 c.c. of solution of ammonia into 50 c.c. of distilled water, and to this mixture add 1 gm. of the best carmine. Stir with a glass rod. After complete solution of the carmine (in about five minutes) add 50 c.c. of a saturated solution of picric acid and let the whole stand in a wide-open vessel for two days. It is then to be filtered. Abundant fungous growth does not diminish the staining power of this excellent medium.

39. *Alum-carmine.*—Dissolve 5 gm. of alum in 100 c.c. of warm distilled water and add 2 gm. of carmine. Boil this mixture ten or twenty minutes and when cold filter; finally, to the clear, beautiful, ruby-red fluid add 2 to 3 drops of liquefied carbolic acid.

40. *Borax-carmine.*—Dissolve 4 gm. of borax in 100 c.c. of warm distilled water; when the solution has cooled add 3 gm. of the best carmine, stirring meanwhile, and then 100 c.c. of 70 per cent. alcohol. At

the expiration of twenty-four hours the fluid should be filtered. It filters very slowly, requiring twenty-four hours or more.

Staining with borax-carmine requires after-treatment with 70 per cent. acid-alcohol, which is prepared by adding 4 or 6 drops of pure hydrochloric acid to 100 c.c. of 70 per cent. alcohol.

Borax-carmine and acid-alcohol should be kept on hand.

41. *Sodium Carminate.*—Dissolve 2 gm. of the pigment in 200 c.c. of distilled water.*

42. *Safranin.*— Dissolve 2 gm. of pigment in 60 c.c. of 50 per cent. alcohol (32 c.c. of 95 per cent. alcohol in 28 c.c. of distilled water). It is to be kept in stock.

43. *Eosin.*—Dissolve 1 gm. of pigment in 60 c.c. of 50 per cent. alcohol. This should be kept in stock.

44. *Congo-red.*—Dissolve 1 gm. of pigment in 100 c.c. of distilled water. From this stock-solution prepare—

(*a*) A $\frac{1}{80}$ per cent. solution: 3 c.c. of stock-solution in 100 c.c. of distilled water.

45. *Vesuvin*, or—

46. *Methyl-violet B.* may be kept in stock in a saturated aqueous solution (1 gm. in 50 c.c. distilled water).

47. *Methylene-blue.*—Dissolve 1 gm. in 100 c.c. of distilled water. This solution keeps well, as does the following, which is required for after-treatment.

48. *Ammonium Picrate.*—Dissolve 3 gm. in 100 c.c. of distilled water.

49. *Orcein.*—Dissolve 1 gm. of the pigment in 100 c.c. of absolute alcohol and add 1 c.c. of pure hydrochloric acid.

50. *Westphal's Alum-carmine Dahlia.*—Dissolve 1 gm. of dahlia in 25 c.c. of absolute alcohol, add 12 c.c. of pure glycerol and 5 c.c. of glacial acetic acid, and pour into this mixture 25 c.c. of alum-carmine (No. 39, p. 24). Preserve in a well-stoppered bottle.

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* *Editor's remark:* Of the carmine stains *alum-cochineal* deserves to be highly recommended. Because of its certainty and of the simplicity of its application it is very useful in the hands of the beginner. It is prepared by boiling 60 gm. of powdered cochineal and 60 gm. of alum in 800 parts of water for about twenty minutes, filtering the decoction, and adding a small piece of camphor or thymol to prevent the growth of mold. It can be kept in stock for a long time.

# II. THE PREPARATION OF MICROSCOPIC SPECIMENS.

## INTRODUCTION.

Very few organs of the animal body are of a structure suitable for microscopic examination without special preparation. They must possess a certain degree of transparency, which is attained either by separating the organs into their elements or by cutting them into thin sections,—that is, either by *isolating* or by *sectioning*. Further, very few organs possess a consistency that, without treatment, allows of the cutting of sufficiently thin sections; they are either too soft, in which case they must be *hardened*, or too hard (calcified), in which case they must be *decalcified*. But fresh objects can be neither *hardened* nor *decalcified* without injury to their structure; both processes must be preceded by treatment which kills the structural elements rapidly and at the same time preserves their natural form. This procedure is called *fixation*. Usually, the preparation of thin sections is possible only after fixation and hardening, followed in some cases by decalcification, of the object. The sections, too, require further treatment; they may be forthwith rendered transparent by means of *clearing media* (which can be also successfully applied in the examination of fresh objects), or they may be *stained* before being made transparent. The staining materials are invaluable agents in microscopic investigations. They can be applied in the examination of fresh and even of living organs. Many of the most important facts have been discovered by means of them. Introduced into the blood-vessels, *injected*, they enable us to trace the branching and course of the finest ramifications.

## § 1. NATURE OF THE MATERIAL.

For the study of the structural elements and the simplest tissues, amphibians (frogs, salamanders) are recommended. The best is the spotted salamander,* the elements of which are very large. For the

* *Editor's remark:* Or the American *Amblystoma*, *Necturus*, etc.

study of organs, mammals should be chosen. In many cases our rodents (rabbits, guinea-pigs, rats, mice), also young dogs, cats, etc., are suitable. Still, no opportunity to secure human organs should be neglected. Perfectly fresh material can often be obtained at surgical clinics. Material may also be had at autopsies, if not made too long after death; with the exception of the mucous membrane of the intestinal tract, which decomposes very quickly after death, many organs can be used.

In general it is advisable to place the organs while yet warm in the fixing fluid. In order to accomplish this the following injunctions must be observed: Fill the bottles selected for the reception of the objects with the appropriate fluid and provide them with a label on which is designated the object, the fluid, the date, and in some cases the hour; then place the dissecting instruments near at hand; then kill the animal.*

## § 2. KILLING AND DISSECTING THE ANIMALS.

In the case of amphibians, cut through the vertebral column of the neck with strong scissors, and destroy brain and spinal cord by means of a needle introduced through the wound into the spinal canal and the cranial cavity. In the case of mammals, cut the throat by a deep incision reaching as far back as the vertebral column, or pour chloroform on a cloth and press it to the nose of the animal.† Small animals, up to the size of four centimeters, and embryos may be placed entire in the fixing fluid; after about six hours the thoracic and abdominal cavities should be opened by incisions. In the dissection, if possible, an assistant should hold the extremities of the animal. Small animals can be extended on cork or wax plates and secured by strong pins thrust through the feet. The organs must be carefully removed. This is best done with scissors and forceps. Crushing or pressing the parts, or taking hold of them with the fingers, must be entirely avoided. Only the edge of the object may be grasped by the forceps. Attached foreign matter—mucus, blood, contents of the intestines—must not be scraped off with the scalpel, but should be removed by slow twirling in the respective fixing fluids.

In the following methods it is not possible to avoid moistening scissors, forceps, needles, glass-rods, etc., with different fluids,—for example,

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* To take parts from the *living* animal is an entirely needless cruelty!

† *Editor's remark:* I prefer to kill medium-sized and smaller animals (rabbits, guinea-pigs, cats, mice, etc.) by placing them under a sufficiently large bell-glass, together with a wad of absorbent cotton saturated with chloroform.

with acids. The instruments should therefore be cleaned *immediately* after using by rinsing in water and drying. Above all, avoid dipping a glass-rod which, for instance, may be contaminated with an acid or a dye into another fluid. Apart from the fact that thereby the reagents will be spoiled, the success of the preparation is, as a consequence, often totally frustrated. Beaker-glasses, watch-glasses, etc., are easy to clean if attended to immediately after using; but if, for example, any staining fluid is allowed to evaporate and dry on them the cleansing then becomes very tedious. Therefore the cleansing of the glasses *immediately* after using should never be neglected; in case there be no time for this, they at least should be placed in water.

All vessels used for isolating, fixing, hardening, staining, etc., must be kept closed, and should not be placed in the sun.

## § 3. ISOLATING.

The process of isolation is accomplished by teasing either the fresh objects or those previously treated with dissociating fluids, which render the teasing partially or wholly unnecessary. It is a difficult task to make a well-teased preparation. Great patience and exact fulfilment of the following directions are indispensable: The needles must be sharp and perfectly clean; they should be previously pointed and polished on a moistened whetstone. The minute object, at the most 5 mm. in length, should be placed in a small drop of the dissociating or mounting medium on a slide and teased,—on a dark background if it is colorless, on a white surface if it is dark or colored. If the tissue is fibrous—for example, a bundle of muscle-fibers—apply both needles at one end and separate the fasciculus along its length into two; in the same way divide one of these bundles into two, and so continue until minute individual fibers are isolated. At times it is difficult to divide the bundle along its entire length; in this case it is often sufficient to divide it for three-fourths of its length, allowing the isolated fibers to remain attached at the one end. The uncovered preparation may be examined with the low power in order to ascertain if the dissection is fine enough.*

The following isolating fluids are recommended:

*For Epithelium.*—Ranvier's one-third alcohol (p. 20) is an admirable dissociating medium. Place small pieces from 5 to 10 mm. in length

* Uncovered preparations lying in a small amount of fluid often appear indistinct, exhibit black borders, etc., errors which may be corrected by the addition of a sufficiently large drop of fluid and the application of a cover-glass.

(*e. g.*, of the intestinal mucous membrane) in about 10 c.c. of this fluid. After four hours (in the case of stratified squamous epithelium after ten to twenty-four hours or later) take out the pieces with the forceps, carefully and slowly, and tap them lightly against a slide upon which a drop of the same fluid has been placed. By this manipulation many isolated epithelial-cells fall off; occasionally shreds are detached, which can be separated into their elements by teasing them. Then apply a cover-glass (p. 44) and examine. If it is desired to stain the object, carefully transfer the entire piece from the alcohol to about 6 c.c. of picrocarmine (p. 24). In two or four hours place the object very carefully in 5 c.c. of distilled water, and in five minutes tap it against the slide, which this time should have on it a drop of diluted glycerol (p. 22). Apply a cover-glass. The preparation can be preserved.

*For Muscle-fibers and Glands.*—A 35 per cent. solution of potassium hydroxid is suitable. Small cubes from 10 to 20 mm. in diameter should be placed in 10 to 20 c.c. of this fluid. In about an hour the objects fall apart into their elements, which may then be lifted out with a needle or a pipet and examined under a cover-glass in a drop of the same lye. The action of diluted potash lye is totally different; examined in a drop of water the elements are rapidly destroyed. If the isolation is not successful, and instead a jelly-like softening occurs, the potash solution is too old. Therefore a freshly-prepared solution should always be used. The preparations, even when successful, cannot be preserved.*

A mixture of potassium chlorate and nitric acid may be used. This is prepared by throwing into 20 c.c. of pure nitric acid enough potassium chlorate (about 5 gm.) so that an undissolved residue will remain on the bottom of the bottle. In from one to six hours, occasionally later, the object is sufficiently dissociated, and should then be transferred to distilled water, in which it should stay for one hour, but may remain for a week without injury. Then the object is placed on a slide, where, in a drop of diluted glycerol (p. 22), it can be easily dissected. If the nitric acid is well washed out the preparation can be preserved and can also be stained under the cover-glass (p. 48). Placing the unteased objects in

* *Editor's remark:* According to S. H. Gage ("Proc. Amer. Soc. Micr.," 1889, p. 36), the action of the caustic potash may be at any time most satisfactorily checked by replacing it with a 60 per cent. solution of potassium acetate, or by the addition of sufficient glacial acetic acid to neutralize the caustic potash and form acetate of potash. After the action of the caustic potash is checked the elements may be preserved indefinitely *en masse* in a 60 per cent. solution of acetate of potash, or after being treated with a saturated solution of alum, in 40 per cent. alcohol or glycerol. After the last treatment the elements may even be satisfactorily stained with hematoxylin or alum-carmine.

picrocarmine (see For. Epithelium) will not be successful, because this staining fluid renders them brittle.

*For gland-tubules* pure hydrochloric acid is excellent. Small pieces about 1 cm. in diameter should be placed in 10 c.c. of the acid and in from ten to twenty hours transferred to about 30 c.c. of distilled water, which must be renewed several times during twenty-four hours. The isolation is then easily accomplished by carefully spreading out the pieces with needles in a drop of diluted glycerol. The preparation can be preserved.

## § 4. FIXATION.

**General Rules.**—(1) For fixation a *large* quantity of the fluid should be used, exceeding the volume of the object 50 to 100 times. (2) The fluid must always be *clear*, and so soon as it becomes turbid must be replaced by fresh fluid. It often becomes turbid within an hour after the introduction of the object. (3) The objects to be fixed should be as small as possible; in general they should not exceed 1 or 2 c.c. Should it be necessary to preserve the object entire (*e. g.*, for subsequent orientation) many deep incisions should be made in it from five to ten hours after placing it in the fixation medium. The object should not lie on the bottom of the receptacle, but should be suspended within it or placed upon a thin layer of cotton- or glass-wool.

1. *Ninety-five per cent. alcohol* is especially suitable for fixing glands, skin, blood-vessels, etc. It acts simultaneously as a hardening medium. Objects fixed in alcohol can be sectioned after twenty-four hours.* Therefore it is well adapted for the rapid preparation of specimens. Special attention should be given to the following details: (1) The alcohol must be renewed in from three to four hours, even though it is not turbid. (2) The objects should not lie in contact with the glass, lest they adhere to it; † therefore they should be either suspended on a thread in the alcohol or placed on a little wad of cotton on the bottom of the vessel.

Weaker alcohol—for example, 90 per cent. alcohol—acts very differently, shriveling the object, and therefore cannot be used instead of 95 per cent. alcohol.

2. Chromic acid is mainly used in two aqueous solutions:

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* One should not too long delay using objects fixed in absolute alcohol, for the elements gradually deteriorate; they should be sectioned in from three to eight days. Sections of objects that have lain only twenty-four hours in absolute alcohol occasionally stain poorly.

† Such areas appear strongly compressed in the sections.

(*a*) As a 0.1 or a 0.5 per cent. solution (p. 20), which is especially suitable for organs that contain much loose connective tissue. This strong solution imparts a superior consistence to connective tissue, but has the disadvantage of making the staining difficult; it is also suitable for the fixation of karyokinetic figures. The objects remain in the chromic-acid solution for from one to eight days, are then washed in running water for from three to four hours, or, if this is not possible, placed for the same length of time in water renewed three or four times, then transferred to distilled water for a few minutes, and finally hardened in alcohol of gradually increased strength (§ 5) and protected from daylight (p. 33, remark *).

(*b*) As a 0.05 per cent. solution, which may be prepared by diluting the 0.1 per cent. solution with an equal volume of distilled water. The application is the same as that of solution *a*, except that the objects remain only twenty-four hours in solution *b*.

Chromic-acid solutions penetrate slowly; accordingly, if the tissue is submitted to the action of the medium for so brief a period as twenty-four hours, only small pieces, 5 to 10 mm. in diameter, should be preserved.

3. *Nitric acid* in a 3 per cent. solution (3 c.c. of concentrated nitric acid [p. 20] to 97 c.c. of distilled water), like the strong chromic-acid solution, is an admirable medium for organs rich in connective tissue. The objects remain for from five to eight hours in this solution and without the previous use of water are transferred directly into alcohol of gradually increased strength for hardening (§ 5).

4. *Kleinenberg's Fluid* (p. 21).—Delicate objects (embryos) should be allowed to remain in this fluid for five hours, more solid parts for from twelve to twenty hours; then, without previous washing in water, they are hardened in alcohols of gradually increased strength (§ 5).

5. *Müller's Fluid.*—The objects remain for from one to six weeks * in a large volume (up to 400 c.c.) of this solution, are then washed in (if possible) running water, rinsed in distilled water, and, finally, hardened in the series of gradually ascending alcohols, under exclusion from daylight (p. 33, remark *). Who does not follow with painstaking conscientiousness the above-specified general rules for fixation will secure imperfect results, for which even otherwise experienced microscopists have held the blameless Müller's fluid responsible.

6. *Zenker's Fluid.*—Metallic instruments must be cleansed imme-

* Objects may be left in Müller's fluid for a longer period—up to six months; often they can then be sectioned and stained without the alcohol hardening.

diately after dipping them into this fluid. The objects should remain in it for from twenty-four to forty-eight hours, allowing about 60 c.c. of the reagent to each one-centimeter cube of tissue, should be washed in running water for the same length of time, rinsed in distilled water, and hardened in the dark in alcohols of gradually increasing strength (p. 33). For the removal of the sublimate precipitates that occur in the tissues add to the 90 per cent. alcohol enough tincture of iodin to impart to the fluid the color of port-wine. The objects remain for from eight to fourteen days in this iodin-alcohol, the color of which rapidly fades, and therefore it requires the daily addition of enough of the tincture of iodin to maintain the desired color.* Finally the objects are transferred to pure 90 per cent. alcohol, which is to be changed two or three times, and in this they may remain for a week or longer. (See also p. 47.)

7. *Osmic-acid Solution* (p. 22).—In using this reagent care must be taken not to inhale the vapor, which is very irritating to mucous membranes. Fixation is accomplished either by immersing very small pieces, up to 5 mm. cubes, in the acid, which is usually employed in a one per cent. solution, of which only a small quantity—from 1 to 6 c.c.—need be used; or by exposing the moist object to the vapor of the osmic-acid solution. For the latter purpose pour 1 c.c. of the 2 per cent. solution into a test-tube about 5 cm. in length and add an equal volume of distilled water; fasten the object by means of quills to the under surface of a cork-stopper, with which the test-tube is then to be securely closed. In from ten to sixty minutes, according to the size of the object, it is removed from the cork and dropped into the fluid in the test-tube. In both cases the objects remain in the acid for twenty-four hours, and during this time the containers must be tightly closed and stood in the dark. Then the objects are taken out, washed for from one-half to two hours in running water, rinsed in distilled water, and hardened in gradually strengthened alcohols (§ 5).

8. *Chromic-acetic osmic acid* (Flemming's solution) (p. 22) is an excellent medium for the fixation of karyokinetic figures. Place the absolutely fresh, still warm pieces, from 3 to 5 mm. in diameter, in 4 c.c. of this fluid, in which they remain for from one to two days, or even longer. Then the pieces should be washed in running water for one hour, better longer, rinsed in distilled water and hardened in alcohols of gradually ascending strength (§ 5). The effect of this mixture on

* If, despite this, the sections still show sublimate precipitates, the latter may be removed by placing the sections in iodin-alcohol for about ten minutes. Then rinse them in pure alcohol, transfer them to the staining fluid, etc. Occasionally, the staining is difficult; this may be remedied by subsequent treatment with diluted potash lye (p. 36, remark *).

the nuclei is different at the periphery of the object than in the interior, where the chromatin networks are more distinct, because at the periphery the osmic acid, which renders the nuclear sap granular and the nuclear reticulum indistinct, acts in its purity.

9. *Platinum-acetic-osmic acid mixture* (p. 22) is very suitable for displaying sharply-defined cell-boundaries. It is used like Flemming's solution.

The fluids that have been used for fixation cannot be employed again, and should be thrown away.

## § 5. HARDENING.

Except when alcohol is used, all the fixing methods necessitate a supplementary process of hardening. The best hardening medium is *alcohol in ascending degrees of strength.* Here, too, the rule is to use abundance of fluid, and to change the alcohol as it becomes turbid or colored.* The exact application is as follows: After the objects have been fixed in one of the previously-enumerated fluids and washed in water,† they are placed, under exclusion of daylight, for twelve hours in 50 per cent. alcohol, then transferred for the same period to 70 per cent. alcohol, and at the expiration of this time to 90 per cent. alcohol in which, after another period of from twenty-four to forty-eight hours, the hardening is completed. In this alcohol the objects may remain for months before their final preparation. The 90 per cent. alcohol employed for hardening should be collected and used for burning, or for hardening liver for embedding.

## § 6. DECALCIFYING.

The objects to be decalcified must not be placed fresh in the decalcifying fluid; they must be previously fixed and hardened. For this purpose place small bones up to the size of a metacarp, teeth entire, and pieces from 3 to 6 cm. long sawed from the larger bones in 300 c.c. of Müller's fluid

* Objects fixed in chromic acid or in Müller's fluid, if not subjected to prolonged washing—and that must be avoided because of incipient decomposition—still yield substances to the alcohol, which with the simultaneous action of daylight appear in the form of precipitates; on the other hand, if the object is kept in the dark no precipitates are formed, and though the alcohol becomes yellow it remains clear. It is on this account that the exclusion of daylight has been recommended above; it is sufficient to place the bottles in a dark part of the room. The 90 per cent. alcohol must be changed once daily so long as it becomes intensely yellow.

† An exception is made in the case of objects that have been fixed in picrosulphuric acid and in 3 per cent. nitric acid. These should be transferred directly from the fixing fluid to the 70 per cent. alcohol, which must be changed several times during the first day.

for from two to four weeks and, after previous washing, harden them in 150 c.c. of gradually strengthened alcohols (§ 5). After the bone has been in the 90 per cent. alcohol for three days or longer it is transferred to the decalcifying fluid—diluted nitric acid, prepared by adding from 9 to 27 c.c. of pure nitric acid to 300 c.c. of distilled water. Large quantities, at least 300 c.c., of this fluid should be used and changed *daily* at first, later every four days, until the decalcification is completed. The process is controlled, and the degree of decalcification ascertained, by thrusting in a needle or by making an incision with a scalpel, which should be at once carefully cleaned. Decalcified bone is flexible, soft, and easily cut. Fetal bones, heads of embryos, etc., are decalcified in weaker nitric acid (1 c.c. of pure nitric acid to 90 c.c. of distilled water) or in 500 c.c. of a saturated aqueous solution of picric acid (p. 21). The process of decalcification requires several weeks in the case of thick bones, from three to twelve days in the case of fetal and small bones.

So soon as the decalcification is completed the bones are washed in running water for from six to twelve hours, and then hardened in gradually strengthened alcohols (§ 5).

It not infrequently happens to beginners that they transfer the bone to alcohol before it is fully decalcified, and then in the attempt to section it they discover that it is not yet ready for use. In such cases the entire procedure of decalcification must be repeated. If the action of the decalcification medium is too prolonged, it eventually leads to the complete destruction of the objects.

## § 7. SECTIONING.

The razor must be sharp, for success in sectioning depends upon the sharpness of the knife. In cutting, the blade must be moistened with alcohol; water is not suitable, because it does not adhere evenly to the surface of the blade. For this purpose dip the knife, at each third or fourth section, into a shallow glass dish containing 30 c.c. of 90 per cent. alcohol, which at the same time serves for the reception of the sections that are cut. The razor is to be held in a horizontal position, grasped lightly, with the thumb on the side of the cutting edge, the fingers toward the back of the blade, the dorsum of the hand directed upward. The object to be sectioned must first have a smooth surface, which is made by cutting off a slice of the necessary thickness with a single movement of the razor. From this surface the sections may now be taken, and should be cut with a uniformly light, not too rapid, movement, as smooth as possible, and of even thinness. The knife must not be pushed, but should be *drawn* through the object, and that this may be

done the portion of the blade adjoining the handle should be first applied to the object. Ten to twenty sections should be made; they may be transferred by means of a needle or by immersing the blade in the alcohol.* Then place the dish on a black surface and search for the best sections. The thinnest sections are not always the most useful; for many preparations, for example, for a section through all the coats of the stomach, thick sections are to be recommended. For a general view, large, thick sections should be prepared; for the study of ultimate structures, thin sections; for the latter purpose small fragments from 1 to 2 mm. on a side are often satisfactory.

If the object to be sectioned is too small to be held with the fingers, it should be embedded. The simplest method consists in placing the object in a cleft in a piece of hardened liver.

Ox-liver or, better, human lardaceous or amyloid liver may be used. The latter may be obtained from the pathologic laboratories. Dog's liver, to be obtained from the physiologic laboratory, is also recommended. The liver should be cut into pieces about 3 cm. high, 2 cm. broad, and 2 cm. thick, and these hardened in 90 per cent. alcohol, which must be changed within twenty-four hours; in three to five days the liver attains the necessary hardness. The embedding is then accomplished by making an incision in one of these pieces from the top half-way down and inserting the object into the cleft thus made. If the object is too thick, furrows can be cut in the liver with a small scalpel and the object fitted into these. The object requires no further staying except, perhaps, binding with thread.

As a rule I embed objects in liver; very thin sections can then be made so soon as one has a certain amount of skill, and this can be easily acquired in the course of a few weeks.

## § 8. STAINING.

Before using a stain it should always be filtered. A small funnel may be made by simply twice folding a piece of filter-paper 5 cm. square and supporting it in a cork frame, which can be made by cutting out a piece 2 cm. square from a cork plate 5 cm. square. The frame is then mounted on four long pins. Such a funnel and frame can be used repeatedly, but only for the same fluid. The sections should not float on the surface of the staining fluid; they should be submerged with needles.

* Very thin sections that are not to be stained or that have been stained in bulk may be transferred directly to the slide by inclining the blade and slipping or rinsing them off.

1. *Nuclear Staining with Hansen's Hematoxylin* (p. 23).—Filter from 3 to 4 c.c. of the staining fluid into a watch-glass and in it place the sections. The time in which the sections stain varies greatly. Sections fixed and hardened in alcohol stain in from one to three minutes. If Müller's fluid was used for fixing, the sections must remain in the staining fluid somewhat longer—up to five minutes.*

From the stain the sections are transferred to a watch-glass containing distilled water, in which they are washed,—*i. e.*, gently moved about with the needle to remove the excess of dye,—and then placed in a glass containing 30 c.c. of distilled water. In this the sections must remain at least five minutes, during which their blue-red color changes to a beautiful deep blue, which becomes the purer the longer (up to twenty-four hours) the sections are allowed to remain in the water. At first the sections have a faded blue tint; usually the differentiation occurs in about five minutes, but sometimes not for hours. When it is completed, certain details can be recognized even by the unaided eye.

Beginners are recommended to leave the sections for different lengths of time—one, three, or five minutes—in the stain, in order to learn the time required to produce successful staining. The chief essential in hematoxylin staining is thorough washing; if the water becomes blue, it must be replaced by fresh. The used stain should be poured back through the filter into the hematoxylin bottle. The watch-glasses should be immediately cleaned.

2. *Nuclear Staining with Alum-carmine* (p. 24).—Filter from 3 to 4 c.c. of the staining fluid into a watch-glass, place the sections in it, and allow them to stain for at least five minutes. The advantage of this dye lies in this, that the sections may be left in it for a longer period without becoming overstained, what is more apt to occur with hematoxylin; a disadvantage is, that alum-carmine is a pure nuclear stain, while in hematoxylin staining the protoplasm too acquires color, a gray or gray-violet tone, and is thereby more easily recognized.

3. *Diffuse Staining.*—For staining the protoplasm and the intercellular substances.

(*a*) *Slow Staining.*—A small drop of neutral carmine solution is

* Sections fixed in the strong solution of chromic acid or in Zenker's fluid, or objects not entirely free from acid, often stain very slowly, occasionally not at all. This defect can be remedied either by keeping the objects from two to three months in 90 per cent. alcohol, which must be changed two or three times during this period; or by treating the sections from five to ten minutes with 5 c.c. of distilled water to which from 3 to 7 drops of 35 per cent. solution of potassium hydroxid have been added. The sections are then to be transferred for from one to two minutes to a watch-glass containing pure distilled water, and from this into the hematoxylin. In from five to ten minutes such sections will also stain.

transferred by means of a glass-rod to a capsule containing 20 c.c. of distilled water, on the bottom of which lies a small piece of filter-paper.* The sections remain over night in this fluid. The paler the rose-color of the fluid, the longer the time required for staining and the more beautiful the result will be. The beginner is always inclined to regard the pale-rose fluid as too dilute to secure good staining, until on the following day the deep pink to red sections teach him better.

This stain can be used alone only in a few cases, but is highly recommended for double-staining. The sections should be stained first with the carmine solution, then with hematoxylin.

(*b*) *Rapid Staining.*—Add 10 drops of a solution of eosin (p. 25) to 3 or 4 c.c. of distilled water. In this the sections remain for from one to five minutes, are then washed in distilled water, and then placed in 30 c.c. of fresh distilled water (see No. 1, p. 36). The stain may be used alone or combined with hematoxylin; in the latter case the whole procedure of hematoxylin staining is to be carried out first, and then that of eosin staining.

4. *Staining of the Chromatin Substance* (for nuclear division).—Place the objects for from five to ten minutes in a watch-glass containing 10 c.c. of distilled water and one drop of pure hydrochloric acid; wash them for one minute in distilled water and transfer them to a watch-glassful of safranin solution (p. 25), in which they should remain five minutes. The sections or membranes are then lifted out with the needle and placed in about 5 c.c. of absolute alcohol for decolorization. When the sections no longer give off much of the dye (usually in from one to two minutes) they are transferred to 5 c.c. of fresh absolute alcohol for one minute, then cleared and mounted (§ 10, 3, p. 45). If the immersion in absolute alcohol is too prolonged, it may lead to total decolorization of the preparation. Failure in staining is usually due to an insufficient amount of acetic acid in the Flemming's solution (p. 22, remark).

5. *Staining in Bulk.*†—(Nuclear staining of the entire object before sectioning.)—The fixed and hardened objects are placed in 30 c.c. of borax-carmine for twenty-four hours if they are small (5 mm. square), for from two to three days if they are larger. From this they are transferred directly to 25 c.c. of acid-alcohol (p. 25); the used borax-carmine may

* If the filter-paper is omitted the sections stain only on the one side.

† *Editor's remark:* It is especially for staining in bulk that *alum-cochineal* (recommended on p. 25, remark) proves very useful. It has the advantage of not overstaining, and does not need in its application a special discharging fluid. Stain the pieces for about twenty-four hours and wash them in several changes of water to remove the excess of stain and the alum; then transfer to alcohol of gradually increased strength.

be returned to the bottle. In a few minutes the acid-alcohol acquires a red color * and must be replaced by fresh, which should be again renewed in about fifteen minutes; this renewal must be repeated until the alcohol no longer becomes red. † The object is then transferred to 90 per cent. alcohol, and if after twenty-four hours it is not sufficiently hardened to be sectioned, it is placed for twenty-four hours or longer in absolute alcohol.

6. *Picrocarmine.*—Double-staining: Nuclei and connective tissue red, protoplasm yellow. Filter about 5 c.c. of the staining fluid into a watch-glass. The length of time in which picrocarmine acts differs greatly for individual objects and can be approximately given only in the special directions. When the staining is completed, the dye is filtered back into the bottle and the object transferred for from ten to thirty minutes to 10 c.c. of distilled water. (The latter procedure is omitted in staining under the cover-glass, p. 48.) If the object, *e. g.*, a section, is to be dehydrated in absolute alcohol (p. 45), it must not be allowed to remain in this reagent longer than from one to two minutes, because the alcohol extracts the yellow stain; or, the decolorization can be prevented by adding a small crystal of picric acid to the absolute alcohol.

Picrocarmine is preferably used in the examination of fresh objects. If the solution is good, very pretty staining is obtained that is improved by subsequent treatment with acidulated glycerol, which renders it crisp and clear.

7. *Nuclear Staining with Anilin Dyes.*—For this purpose the best anilin dyes are *vesuvin* and *methyl-violet, B* (p. 25). Filter 5 c.c. of the staining fluid into a watch-glass; in this place the sections, which acquire a very dark color in from two to five minutes; they are then washed in, distilled water and transferred to a watch-glass containing absolute alcohol, in which they give off the dye abundantly. In a few minutes, from three to five, the sections become paler, and individual parts (*e. g.*, the glands of the skin) can be detected by the unaided eye. The sections are now to be transferred to another watch-glass containing 5 c.c. of absolute alcohol, and in about two minutes they may be cleared and mounted. The result is a very beautiful permanent nuclear stain. A disadvantage lies in the necessity for using so much absolute alcohol.

*Safranin* can be similarly employed. The sections stained for five

* Preparations fixed in Müller's fluid often give off very little dye.

† This may require from one to three days; during the first day the fluid should be changed every two hours, subsequently every four hours. If you wish to be economical, take a needle and gently push the object out of the area of red fluid in which it lies into uncolored portion of the alcohol.

minutes are washed for thirty seconds in a watch-glass containing 95 per cent. alcohol and then transferred to absolute alcohol, which must be replaced by fresh so soon as it becomes intensely red. In from five to fifteen minutes—the time varies according to the thickness of the sections—they are sufficiently decolorized and are then to be cleared in oil and mounted in damar-varnish (p. 45).

8. *Methylene-blue for Staining Axis-cylinders.* — This method is applicable only to perfectly fresh preparations. Prepare a $\frac{1}{15}$ per cent. solution, which may be done by adding 1 c.c. of a 1 per cent. solution (p. 25) to 15 c.c. of distilled water. The fresh preparation is treated on the slide with a few drops of the diluted staining fluid, and meanwhile protected with a watch-glass, which must not be so applied as to make an hermetic cover, since the access of atmospheric air is necessary to the success of the staining. The reaction occurs in from one to one-and-a-half hours, and can be rendered more certain by gently moving the preparation to and fro. In order to prevent the drying of the preparation during this period, a drop of the diluted staining fluid or of normal salt solution should be added from time to time. When the staining is done, a cover-glass should be applied. The result is a beautiful blue coloration of the axis-cylinders. Other elements often are stained, the nuclei, connective-tissue fibers, etc., and with more prolonged action of the reagent also the medullary sheaths of the nerves. The preparation may be preserved as follows: replace the staining fluid with a drop of ammonium picrate solution (p. 25) according to the method given on p. 48; this converts the blue color to violet; then place a drop of glycerol at the edge of the cover-glass, and it will gradually take the place of the evaporating water of the ammonia solution, and thus make the specimen permanent.

After eighteen to twenty hours secure the cover-glass with cement (p. 45). The preparations must not be exposed to sunlight, in which they fade; in any case they soon lose their original beauty.

9. *Delafield's Hematoxylin for Staining Mucus.*—Filter three drops of this fluid (p. 23) into a watch-glass containing 25 c.c. of distilled water. In this dilute solution the sections (preferably of objects fixed in Flemming's mixture*) are placed and remain for two or three hours. Usually at the end of this period the mucus (*e. g.*, in the goblet-cells) is stained an intense blue, which can be ascertained by examining with low magnification the sections as they lie in the solution. It is often neces-

* Preparations that have been fixed in Müller's and in Zenker's fluid are also suitable for mucus-staining.

sary for the sections to remain in the solution for a longer time. Then they are washed for one minute and mounted in damar, according to the rules given in §10, 3, p. 45. The nuclei also stain blue. Very pretty pictures are obtained by a combination with safranin and picric acid, as in No. 10.

10. *Triple-staining* is accomplished in the following manner: The sections stained in Delafield's hematoxylin are placed for five minutes in safranin (p. 25) and then transferred to 5 c.c. of absolute alcohol, which must be changed twice within fifteen minutes. The sections are next placed for one minute in 5 c.c. of absolute alcohol, to which five drops of a saturated alcoholic solution of picric acid have been added (1 gm. of picric acid to 15 c.c. of absolute alcohol), washed for thirty seconds in pure absolute alcohol, and mounted in damar (§ 10, 3, p. 45).

Result: mucus, blue; nuclei, red; protoplasm and fibers, yellow.

11. *Staining of Elastic Fibers.*—Sections of objects fixed in any medium are placed in 5 c.c. of a solution of orcein (p. 25), and after about fifteen hours are decolorized in 10 c.c. of acid-alcohol (see Borax-carmine, p. 24), in which they remain for from twenty to sixty minutes, according to their thickness. If they are left too long in the acid-alcohol the dye is extracted from the fine elastic fibers. Therefore the process of decolorization should be controlled by frequent examination of the sections. In successful preparations the elastic fibers appear brown-red on a light background.

12. *Silver Staining.*—For the exhibition of cell-boundaries and the staining of intercellular cement-substance.*

The use of metallic instruments must be avoided; glass-rods should be employed, and quills instead of pins.

The object is immersed for from one-half to ten minutes, according to its thickness, in 10 to 20 c.c. of a 1 per cent. or weaker (see Special Technic) solution of silver nitrate (p. 22), which meanwhile becomes milky and turbid; it is then removed with glass-rods, washed, placed in a porcelain capsule containing 100 c.c. of distilled water, and exposed to direct sunlight; in a few minutes a faint brown coloration appears—the sign of a successful reduction. So soon as the object has become a deep red-brown (usually in from five to ten minutes) it is taken out, placed in a watch-glass containing distilled water to which a few grains of common salt have been added, and at the end of five or ten minutes transferred to 30 c.c. of 70 per cent. alcohol, and stood in the dark; in

* The cross-striations that appear in different tissues and organs, when treated with silver nitrate, particularly in nerve-fibers, blood-vessels, cartilages, etc., are artefacts.

from three to ten hours the 70 per cent. should be replaced by 90 per cent. alcohol. The immersion in the silver solution should be done under exclusion of sunlight; the reduction, on the other hand, should be undertaken only with sunlight.* If the sun does not shine, the object, after treatment with the silver solution and washing in distilled water, is to be preserved in the dark in 30 c.c. of 70 per cent. (later 90 per cent.) alcohol, and in this exposed to sunlight at the earliest opportunity.

13. *Golgi's "black" reaction* for demonstration of the elements of the nervous system.†

This method unites fixing and staining. The objects must be as fresh as possible, and in general their diameter should not exceed 4 mm. It is not easy to cut fresh brain into pieces of this size without bruising the delicate tissue; therefore place larger pieces (up to 3 cm. cubes) in a small glass jar containing freshly prepared Golgi's mixture (p. 21), which is to be covered and stood in the dark (in winter it must be put in an oven having a temperature of about 25° C.). In from one to two hours the pieces can easily be cut into slices about 4 mm. in diameter. The quantity of Golgi's fluid to be used is regulated by the number of the slices, each slice requiring about 10 c.c. of the mixture. In from two to six days, less often fifteen days,‡ the slices are taken out, quickly washed for a couple of seconds in distilled water, gently dried with filter-paper, and placed in 0.75 per cent. silver solution (30 c.c. of the 1 per cent. solution [p. 22] plus 10 c.c. of distilled

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* The reduction takes place in ordinary daylight, but slowly, and yields less satisfactory results.

† *Editor's remark:* In American laboratories a modification of Golgi's method by Cox is often used with excellent results. This modification is particularly recommended to beginners, because it is very simple and nearly always successful. In its application the following directions should be observed: Put small cubes, 2 cm. or less, of the organs of the central nervous system of adult or newborn animals for from six to ten weeks into the Cox-Golgi mixture, the formula of which is given on p. 21 (No. 15), using 10 to 20 times the volume of the object treated. Change the fluid at the following intervals: after twenty-four hours; three days; eight days; fifteen days; twenty-one days; thirty days. The objects should remain in the mixture until they are to be sectioned, and will keep in good condition for about ten months. Then transfer them directly into 95 per cent. alcohol for one hour; into alcohol-ether (equal parts) for a half hour; into thin celloidin solution (in alcohol-ether) for one hour. Mount on a block with thick celloidin solution (see Microtome Technic) and harden in 80 per cent. alcohol for from one to two hours. Cut at once sections from 50 to 100 $\mu$ thick; clear them in a mixture of xylol, three parts, and carbolic acid, one part, in which they may remain for weeks without injury. Mount in balsam and *cover the sections with a cover-glass.* In time the specimens thus preserved are not infrequently marred by the appearance of corrosive crystals, but the impregnation of the elements of the nervous tissue remains intact.

‡ See Special Technic.

water, and for each piece 10 c.c. of this fluid).* A brown precipitate immediately surrounds the pieces. They should be left in the silver-solution for two days (which need not stand in the dark and must not be placed in the oven), and they may remain in it for six days without injury; they are then placed for from fifteen to twenty minutes (not longer) in 20 c.c. of absolute alcohol, then embedded in elder-pith (or in celloidin, see Microtome Technic) and cut into thick sections (p. 34).

Each section should be examined, without a cover-glass, with the low power, in order to ascertain its usefulness; if it is good, it is placed for from one to two minutes in a watch-glass containing absolute alcohol, then in creosote for two minutes, and in oil of bergamot for two minutes; from this it is transferred for a few seconds to xylol, then placed upon the slide. Finally the xylol is removed by light pressure on the section with clean filter-paper and the preparation covered with a few drops of Canada-balsam diluted with xylol. A cover-glass must *not* be applied, because it would prevent evaporation of the moisture in the section, which when retained destroys the Golgi preparations. Not infrequently —especially when the xylol has not been satisfactorily removed—the Canada-balsam gradually withdraws from the preparation, which in consequence appears spoiled, but may be fully restored by the application of a fresh drop of balsam. At first the preparation should be examined with the low-power objective; when the balsam has become dry the high power may be used.

The results obtained by this method, when successful, are admirable; single elements of the nervous system (never all), occasionally also blood-vessels, lymph-vessels, connective-tissue fibers, secretions, muscle-fibers, and epithelial-cells stand out in full relief—black on a light background. But the method is subject to various accidents. Almost invariably the best sections are disfigured by black precipitates; these occur chiefly at the edges of the preparations; in order to avoid them it has been suggested that a layer of coagulated blood be applied to the fresh object. Very often the reaction fails entirely (especially when the action of the Golgi mixture was too prolonged); then the so-called "double method" may lead to success. If the first sections show nothing, the objects should be again treated with Golgi's fluid for from twenty-four to thirty-six hours, and for the same length of time with the silver solution. A second failure may be occasionally crowned with success by a second repetition of the procedure. In the application of Golgi's method practice and patience are important factors.

* The used Golgi mixture is to be thrown away.

Preparations to which the foregoing method has been applied can be "fixed." For this purpose the sections are transferred from the alcohol to a mixture of 10 c.c. of absolute alcohol* and 20 c.c. of the diluted solution of hydrochinone (p. 21); in this they remain five minutes, during which they become dark gray to black. When the reduction is completed, the sections are directly transferred for from ten to fifteen minutes to a glass containing 70 per cent. alcohol. In this they become paler, then are placed in the sodium-hyposulphite solution (p. 21) for five minutes, and finally in a large capsule containing distilled water, in which they must remain at least twenty-four hours or longer. The preparations that have been thus "fixed" can be mounted, like other preparations, under a cover-glass, and staining with alum-carmine or hematoxylin is sometimes successful.

14. *Gold Staining*, for the demonstration of nerve terminations.—Steel instruments must not be used; all manipulations in the gold solution are to be performed with rods of glass or wood. Put 8 c.c. of a 1 per cent. gold-chlorid solution and 2 c.c. of formic acid into a test-tube and heat the mixture to the boiling-point; let it boil up three times. Into the *cooled* mixture very small cubes of tissue (at most 5 mm. square) are placed for one hour, during which they must be kept in the dark; then they are washed in distilled water and exposed to the light in a mixture of formic acid, 10 c.c., and distilled water, 40 c.c. Sunlight is not necessary. The reduction takes place slowly, often not until after twenty-four or forty-eight hours, the exterior of the cubes meanwhile assuming a dark-violet hue. When the reduction is completed, place the tissue in 30 c.c. of 70 per cent. alcohol and on the following day in an equal quantity of 90 per cent. alcohol, in which, to hinder further reduction, they must remain in the dark for at least eight days before their final preparation.

## § 9. INJECTING.

The filling of the blood- and lymph-vessels with colored masses is a special art that can only be acquired through much practice. The knowledge of the many little devices employed can scarcely be attained through didactic teaching, however painstaking and explicit. Here practical instruction is indispensable. Accordingly, since this book is intended for beginners, it seems wise to refrain from entering upon a detailed account of the technic of injecting.

* Excess of alcohol produces a precipitate, which can be promptly removed by the addition of more hydrochinone solution.

He who desires to attempt injecting must have an accurately-closing, smoothly-working hand-syringe, provided with cannulæ of different sizes. For an injecting mass Berlin blue (Grübler) is recommended—3 gm. dissolved in 600 c.c. of distilled water. It is advisable to begin with the injection of single organs—for example, the liver, which is preferable because it gives useful results, even though the blood-vessels are but partially filled. The injected object should be fixed for from two to four weeks in Müller's fluid (p. 31) and hardened in gradually strengthened alcohols (p. 33). The sections should not be very thin.

## § 10. MOUNTING AND PRESERVING OF THE PREPARATIONS.

The finished sections and other objects prepared according to the foregoing methods, in order that they may be examined under the microscope, are finally mounted on a slide and covered with a cover-glass. The media in which the sections are mounted are: (1) *water;* or, if the section is to be cleared and preserved, (2) *glycerol;* or (3) damar-varnish.

The transfer of the object to the slide is usually done in this way: a small drop of a suitable fluid is placed on the middle of the slide; the section is then taken up on the section-lifter, and with the aid of the needle slipped off onto the slide. Very thin sections are better lifted on the end of a glass-rod, and by rolling of the latter transferred to the slide. When the section is smoothly mounted, it is covered with a cover-glass.* The latter must be grasped by its edges, not by its surfaces. It should be taken in the left hand, one edge placed in contact with the slide, and then, supported on its under surface by a needle held in the right hand, slowly lowered upon the preparation. It is simpler to suspend a drop of the mounting medium from the inferior surface of the cover-glass and then to let it softly fall upon the preparation. The fluid in which the section is mounted must occupy the entire space between cover-glass and slide. If the amount of fluid is insufficient, another drop should be placed at one edge of the cover-glass by means of a glass-rod. If there is too much fluid—and here the beginner

* Examinations with low powers, without a cover-glass, are permissible only for the most superficial orientation; *e. g.*, to ascertain if an object has been sufficiently teased. In all other cases the cover-glass is indispensable. In order to convince one's self of this an uncovered section should be examined, then covered with a cover-glass, and examined again. Many a good preparation that one neglects to cover appears useless. Examinations with high-power objectives without a cover-glass are in general not allowable.

strives to perpetrate impossibilities—the excess which has escaped from beneath the edges of the cover-glass should be absorbed with filter-paper. *The upper surface of the cover-glass must always be dry.* Small air-bubbles under the cover-glass may be removed by cautiously raising and lowering the cover-glass several times with the needle (see further, p. 46).

1. The examination of the unstained and the stained sections in *water* or *normal salt-solution* should never be neglected, since many structural peculiarities—for example, connective-tissue formations—stand out distinctly in these media, which under the clearing influence of glycerol or damar-varnish almost entirely elude observation. Preparations mounted in water or salt-solution cannot be preserved.

2. Preparations mounted in *glycerol* can be preserved; in order to prevent the shifting of the cover-glass it should be secured with cover-glass cement (p. 23). The edge of the cover-glass must be perfectly dry; this is an indispensable preliminary condition, because the cement adheres only to a dry glass surface. The drying is accomplished in this wise: remove the excess of glycerol surrounding the cover-glass with filter-paper and then, with a cloth moistened in 90 per cent. alcohol and turned over the finger-tip, carefully wipe the slide clean all around the cover-glass without disturbing the latter. Now heat a glass-rod and dip it into the hard cement;* place a drop at each corner of the cover-glass and trace a continuous border from 1 to 3 mm. wide, in such a way that one edge rests on the cover-glass, the other on the slide. Finally, heat the rod again and smooth the surface of the band of cement.†

Preparations mounted in glycerol often do not become transparent until the second or third day. Hematoxylin and other dyes soon fade in it; picrocarmine and carmine, on the contrary, are permanent.

3. The mounting of objects in *damar-varnish* is the most popular preserving method. In comparison with glycerol it has the advantage of keeping the colors, but has one disadvantage; it clears more vigorously than diluted glycerol, and thus renders many delicate structures completely invisible. Sections in alcohol or water cannot without further treatment be mounted in damar-varnish,—they must be previously dehydrated. For this purpose the sections are lifted with a needle (very thin sections with needle and section-lifter) and placed in a covered watch-glass containing

* Glass-rods fracture very easily in this procedure, nevertheless are preferable to metal rods, because the latter cool too quickly. The fracturing can be prevented in a measure by heating the glass-rod to red heat, meanwhile turning it continuously; only rods insufficiently annealed break when they are dipped into the cement.

† *Editor's remark:* King's fluid cover-glass cement (p. 23, foot-note) is to be applied with a small brush.

5 c.c. of 95 per cent. alcohol. In making this transfer, as little as possible of the water should be allowed to adhere to the section. If a section-lifter is used the water clinging to it should be absorbed with filter-paper; if the sections are lifted on a needle the water can be removed by bringing the filter-paper into gentle contact with them. Thin sections remain in the 95 per cent. alcohol two minutes; thick sections, ten minutes or longer.* Then the sections are transferred for clearing to a watch-glass containing 3 c.c. of oil of bergamot, as much as possible of the alcohol being removed with filter-paper before placing them in the clearing agent.† If the watch-glass is placed on a black background the effect of the oil can be watched, and it will be seen that the sections gradually become transparent. Care must be taken not to breathe into the watch-glass, or the oil of bergamot will immediately become turbid. If some areas of the section do not become transparent within two or three minutes (such areas appear white and opaque in direct light, black-brown in transmitted light), this indicates that the section is not dehydrated and it must be put back into the absolute alcohol. When the clearing is completed the section is transferred to a dry slide, the superfluous oil‡ absorbed with filter-paper or carefully wiped up || with a linen cloth turned over the index-finger, and a cover-glass, from the under surface of which a drop of damar is suspended, applied. If several sections are to be mounted under one cover, arrange them close together with a needle; then, by means of a glass-rod, apply a thin, even layer of damar to the under surface of the cover-glass and place it on the sections. Large air-bubbles are driven out by placing a small drop of damar at the edge of the cover-glass; on the following day it will be seen that the air-bubbles have retreated from beneath the cover. Small air-bubbles disappear spontaneously and may be neglected.

It not infrequently happens to beginners to discover that the damar becomes turbid and finally renders the entire preparation, or parts of it,

* Beginners are recommended to transfer the sections from the water to 5 c.c. of 90 per cent. alcohol, and then to place them in an equal quantity of 95 per cent. alcohol.

† Thin sections may be transferred from the 95 per cent. alcohol directly on to the slide, the superfluous alcohol wiped off, and a drop of bergamot oil applied. At first the oil will withdraw from the section and must be led back with the needle; when the clearing is completed, which can be ascertained under the microscope with the low power, as much as possible of the oil should be wiped up and a cover-glass with a drop of damar applied. When examining uncovered sections lying in oil both oil and sections often become clouded by the moisture exhaled in breathing; in this case drain off the clouded oil and add a fresh drop.

‡ The oil in the watch-glass that has been used for clearing may be returned to the bottle.

|| The removal of the oil is most readily accomplished by inclining the slide and then wiping it.

untransparent. This is due to incomplete dehydration. If the clouding is slight, which under the microscope is seen to consist of minute drops of water, a gentle warming of the slide is often sufficient to remove it. In the case of much-clouded preparations, place the whole slide in turpentine for half an hour; then carefully lift off the cover-glass, place the section for two minutes in turpentine, in order to dissolve off the adherent varnish, and then dehydrate in 4 c.c. of absolute alcohol, which should be changed in five minutes; clear in oil of bergamot and mount in damar.

The damar-varnish dries very slowly, therefore the slides must not be stood on edge, but be kept in a horizontal position.

The series of processes through which a fresh object must pass until it is preserved as stained sections is a very long one. When, for example, the directions in the Special Technic require: "Fixation in Zenker's fluid, hardening in gradually strengthened alcohols, staining of sections in carmine and hematoxylin, mounting in damar," the procedure is as follows:

1. Place the fresh object, about 1 cm. in diameter, in 60 c.c. of Zenker's fluid * for twenty-four hours.
2. Wash in (if possible running) water for twenty-four hours.
3. Place in 20 c.c. of distilled water for about fifteen minutes.
4. Transfer to 50 c.c. of 50 per cent. alcohol for twenty-four hours; from now on the object is to be kept in the dark (see p. 33, remark*).
5. Transfer to 50 c.c. of 70 per cent. alcohol for twenty-four hours.
6. Transfer to 50 c.c. of 90 per cent. alcohol and tincture of iodin for from eight to fourteen days, daily adding tincture of iodin (p. 32).
7. Transfer to pure 90 per cent. alcohol, which is to be changed two or three times.

The object thus fixed and hardened can be sectioned at once or may remain indefinitely in the 90 per cent. alcohol, which during this period should perhaps be changed once more.†

8. Transfer the sections from the alcohol (p. 36) to 20 c.c. of dilute carmine solution for twenty-four hours.
9. Place them in 5 c.c. of distilled water for ten minutes.
10. Place them in 5 c.c. of hematoxylin for five minutes.

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* The quantities named are calculated only for this 1 cm. cube; for several or for larger objects more fixing and hardening fluid must be used.

† The following quantities are intended for from three to six sections; for a larger number of sections the quantity of the absolute alcohol in particular should be increased.

11. Place them in 30 c.c. of distilled water for from ten minutes to two hours.

12. Place them in 5 c.c. of 95 per cent. alcohol for ten minutes.

13. Place them in 3 c.c. of bergamot oil for two minutes.

14. Mount in damar.

## § 11. EXAMINATION OF FRESH OBJECTS.

I have placed this method last because it is the most difficult and presupposes a somewhat practised eye. This practice is most readily acquired by previous examination of prepared (hardened, stained, etc.) objects; having once clearly perceived and studied peculiarities of structure, it is then not difficult to detect them again in fresh objects, even though the majority of the details leave something to be desired in point of distinctness. The following instructions should be observed:

The slide and the cover-glass must not be oily. They should be cleansed with alcohol and dried with a perfectly clean cloth.* Then transfer *one* drop of a 0.75 per cent. salt solution (p. 19) to a slide, place in it a *small* piece of the object to be examined and cover it with a cover-glass. Pressure must be carefully avoided; if the structures are very delicate, support the cover-glass on two strips of thin paper placed at the sides of the object. If the object requires no further treatment, the cover-glass should be sealed with paraffin to prevent evaporation. Melt a small piece of paraffin on the blade of an old scalpel and let it flow, not from the tip but from the edge, on to the rim of the cover-glass; gaps that may occur in this frame of paraffin can be closed with the re-heated scalpel. In most cases the influence of certain reagents (acids, alkalies, stains) is studied directly under the microscope. It is then necessary to remove a portion of the medium in which the object happens to be mounted (in the present instance the salt solution) and to replace it by another fluid. For this purpose place a drop of picrocarmine at the right edge of the cover-glass. Should the drop not touch the edge of the cover-glass, do not incline the slide, but lead it with a needle to the appropriate position. It may now be seen that a little of the staining fluid mingles with the salt solution, but does not properly flow under the cover-glass. In order that this may occur, place at the left edge of the cover-glass a little piece of filter-

* For removing the oil from new cover-glasses, heating them on a piece of sheet-iron for five minutes over the flame of a Bunsen burner is recommended.

paper * and presently the picrocarmine will be seen to diffuse under the cover-glass and occupy the entire area.† Then remove the filter-paper and let the stain act; when the staining is completed—this can be ascertained under the microscope—place at the right edge of the cover-glass a drop of diluted glycerol to which, in picrocarmine staining, as much acetic acid is added as will drop from a steel needle (hence a very small drop), and again apply the filter-paper to the left edge of the cover-glass. In this way a whole series of fluids can be passed through beneath the cover-glass, and their action on the tissues studied. Some of these fluids—for example, picrocarmine—must remain in contact with the objects for a very long time if they have been previously fixed with osmic acid. In this case evaporation is prevented by placing the object in a *moist-chamber*. For the construction of the moist-chamber a porcelain plate and a small bell-glass 9 cm. in diameter are required. Pour water into the plate, to the depth of 2 cm. and stand in the middle a small glass-dish; on the latter place the slide with the preparation and cover the whole with the glass-bell, the free edge of which must be submerged in the water.

## § 12. STORING OF PERMANENT PREPARATIONS.

The finished preparations should be promptly labeled. Labels of cardboard about 1.2 mm. thick, glued to the slide with fish-glue (isinglass) are preferable to those of gummed paper; the slides can then be placed one upon the other without injury to the preparations. The labels should be as large as possible (2 cm. square for slides of English form) and should bear the name of the animal, of the organ, and if possible a brief statement of the methods used. Of the cases ‡ for storing the preparations only such should be chosen in which the slides lie flat, not those in which they stand on edge.

* Cut a strip 4 cm. long and 2 cm. broad, fold it square and place the paper tent thus formed on the slide, so that one of the narrow ends, which must be perfectly straight, touches the left edge of the cover-glass.

† After the first drop has penetrated, place two or three additional drops at the right edge of the cover-glass.

‡ The best and cheapest cases are kept by Th. Schröter, Leipzig, Windmühlenstr. No. 46. I recommend for box form, *pattern O* (for about 300 slides), price 2 *M.* (50 cents); for tray form, *P*, with flat covers for 10 to 20 slides (according to size), price 45 Pfennige (about 12 cents). The tray form has the great advantage of allowing all the specimens to be seen at once. In the United States, Schröter's boxes and trays are supplied by King & Co., New York, and other dealers.

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## III. MANAGEMENT OF THE MICROSCOPE.

In conformity with the position taken in the introduction, an exhaustive description of the optic and mechanical parts of the microscope cannot be entered upon here. Fig. 1 will recall to the reader the usual names of the several parts of the microscope.

The first requisite in the use of the microscope is perfect cleanliness of all its parts (see also p. 17). The surface of the mirrors, objectives, and oculars should not be touched with the fingers. The objective should be held with the lower end directed toward the window and the clearness of the reflected image thus tested. Foreign matter on the ocular can be detected by rotating the latter in the tube, when anything that is adherent will revolve.

After the ocular has been placed in the upper end of the draw-tube and a low-power objective screwed on the lower end of the tube (or on the revolver, if used), the field of view of the microscope should be illuminated with light reflected from a suitable source by the concave mirror placed below the stage. This is best accomplished by moving the mirror tentatively in all directions (with the diaphragm widely open, and the front-lens of the objective about 1 cm. above the level of the stage) till the eye, looking simultaneously through the eye-piece into the microscope, sees the field of view brightly and uniformly illuminated.* The concave mirror should be used with dry lenses, except when a substage condenser is employed.

The light reflected from a white cloud or from a white window-blind illuminated by the sun is recommended; less desirable but still useful as a source of light is the blue sky. Direct sunlight must be avoided. In using artificial illumination the light should be taken from the inner surface of a white lamp-shade, not directly from the flame. A screen of blue

* The rays of light reflected from the mirror in this position pass perpendicularly through the object on the stage. This is called "*central illumination.*" For distinguishing slight differences of level between adjacent parts of an object it is of advantage to use "*oblique or lateral illumination,*" to obtain which the mirror is moved to the side so that the rays reflected from it strike the object obliquely. When lateral illumination is used the diaphragm and the cylinder in which it is mounted must be removed, so that the opening in the stage shall be as large as possible.

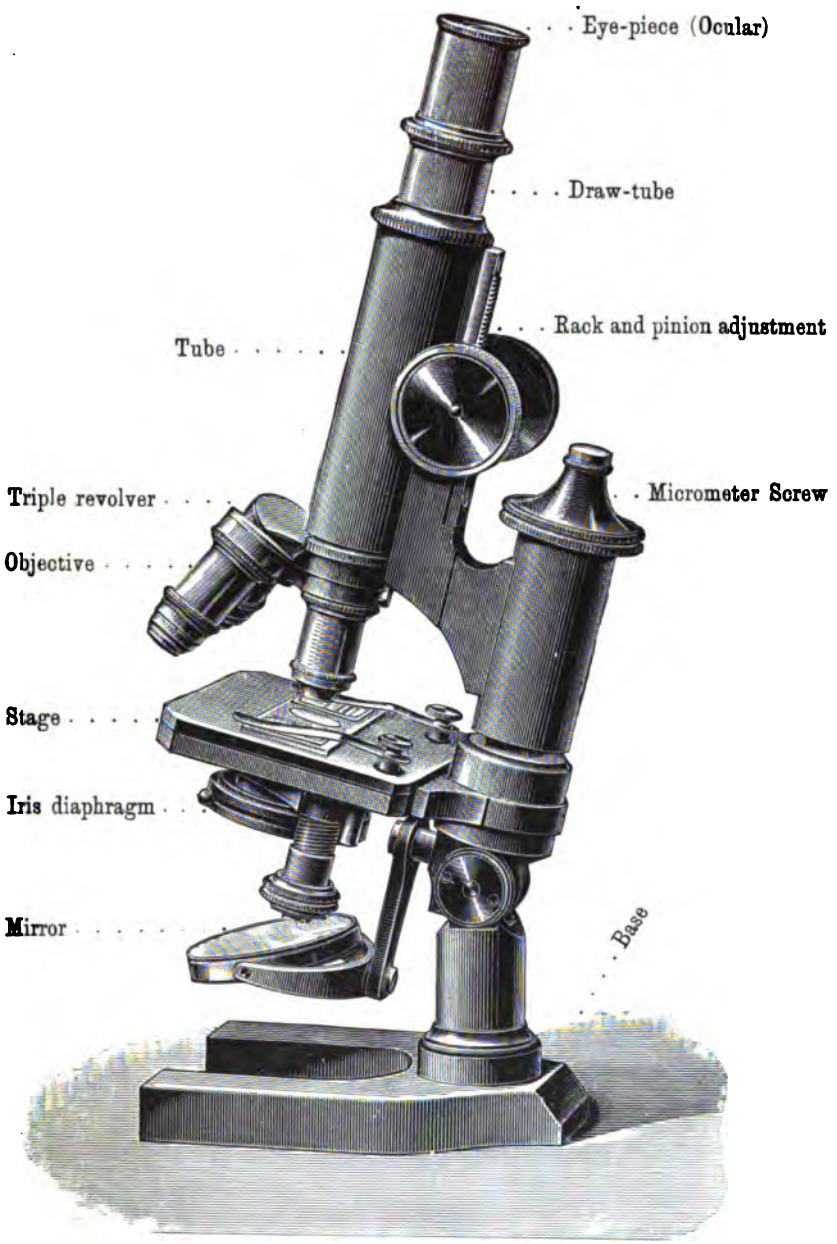


FIG. 1.—LEITZ MICROSCOPE. STAND II (½ actual size).

glass placed between the mirror and the source of light, or between the mirror and the object, agreeably subdues artificial light, without essentially injuring the definition of the image. It is obvious that the micro-

scopist should not sit in direct sunlight; the instrument should be placed about a meter from the window.

Having secured the light the examination may begin. *Always examine first with the low-power, then with the high-power objective; do not use strong oculars*, they narrow and darken the field of view and render the examination much more difficult.* The low- and the medium-power oculars (Leitz, Oc. I) of the usual outfit supplied with the microscope answer for the great majority of cases.

The increased magnification obtained by drawing out the draw-tube is seldom necessary. With low-power lenses a diaphragm having a large opening should be used; with high-power lenses, a diaphragm having a small opening. In focusing the object, the coarse adjustment by rack and pinion is used first; the objective is placed near to the object, but at a distance greater than its focal length, and then, with the eye applied to the ocular, the tube should be gradually lowered until the indistinct outlines of the image appear, which is then brought into distinct view by means of the fine adjustment or micrometer-screw. The left hand should hold the slide, while the right should remain at the micrometer-screw. Since only the points lying in a single plane of the object can be in focus and distinctly seen at one time, the preparation must be examined with slight raising and lowering of the tube, that is, with change of focus by gently turning the micrometer-screw. In using the microscope the habit should be formed of keeping both eyes open.

One should never neglect to examine the preparations with a hand-lens. For this purpose the oculars (*e. g.*, Leitz, Oc. III) can be used. The mounted specimen is held with the cover-glass side toward the light; the upper or back-lens of the ocular is placed directly against the slide, the eye applied to the lower or front-lens.

## DRAWING.

An invaluable aid to study is the drawing of the microscopic object. The power of observation is made considerably keener, and many details which would be otherwise completely overlooked are discovered while the sketch is in progress. Even the most attentive examination cannot replace the advantage which drawing yields. Those who have little practice in drawing should nevertheless try to sketch the preparations under both low- and high-power objectives. For this purpose the

* The majority of the preparations from which the illustrations in this book were taken were examined and sketched with weak oculars.

drawing-paper should be on a level with the stage, the left eye applied to the microscope, the right eye directed to the paper and the pencil-point. At first this is somewhat difficult, but a little practice will soon give the necessary facility.

## MEASUREMENT.

For this purpose an ocular-micrometer and a stage-micrometer are used.*

The latter is laid on the stage of a microscope provided with an ocular-micrometer, and the number of divisions of the ocular-micrometer which corresponds to one part of the stage-micrometer is counted.† The dimensions of the spaces of the stage-micrometer being known, the size of the object, which with a given magnification will occupy one or more of the divisions of the ocular-micrometer, is easily calculated. The following illustrations may render the manipulations intelligible:

Ocular I, and draw-tube pushed in, 5 divisions of the ocular-micrometer correspond with 1 division of the stage-micrometer. Each division of the stage-micrometer used = $\frac{1}{20}$ mm. Hence 5 divisions of the ocular-micrometer = $\frac{1}{20}$ (0.05 mm.), and 1 division of the ocular-micrometer = 0.01 mm. If, then, any microscopic object, *e. g.*, a striated muscle-fiber, the diameter of which is to be measured with this magnification, occupies 4 divisions, the fiber is 0.04 mm. broad.

It is often difficult, especially with low magnification, to count the fine divisions of the ocular-micrometer. This can be more easily done by noting the longer lines marking every fifth or tenth division. For instance, with Leitz Objective 3, Ocular I, and the draw-tube drawn out, 40 divisions of the ocular-micrometer correspond with 5 divisions of the stage-micrometer. Therefore, 40 divisions = $\frac{5}{20}$ mm. = 0.25 mm., and 1 division of the ocular-micrometer with this magnification = 0.0062 mm., 2 divisions = 0.0124 mm., and so on.

With Leitz Objective 7, Ocular I, and draw-tube pushed in, 30 divisions of the ocular-micrometer correspond with 1 division of the stage-

* Some ocular-micrometers (Leitz) are made to rest upon the diaphragm inside the ocular; others (Seibert) to be inserted through a lateral opening; or, in some cases, special oculars (Zeiss) for measuring are made for the microscope. The actual size of the divisions of the ocular-micrometer need not be known. The stage-micrometer is a glass slide on which 1 mm. with 100 subdivisions is engraved. Instead of this a second ocular-micrometer, which usually contains a mm. with only 20 divisions, may be used. Measurements made with this are not so accurate, but the errors are so insignificant that they scarcely need consideration.

† Beginners often find it difficult to focus the lines on the stage-micrometer; faint or oblique illumination of the object makes it easier to detect the lines.

micrometer; 30 divisions = 0.05 mm., 1 division = 0.0017 mm., or 17 $\mu$. Finally, with Leitz Objective 7, Ocular I, and draw-tube drawn out, 40 divisions of the ocular-micrometer = 1 division of the stage-micrometer. Therefore, 40 divisions = 0.05 mm., 1 division = 0.0012 mm., or 12 $\mu$.

It is advisable, if one has many microscopic measurements to make, to prepare a table for each magnification used, in which the equivalent values of 1, 2, 3 . . . . . 20, 30, 40 . . . . . 100 scale divisions of the ocular-micrometer are given. It must be emphasized that the foregoing calculations by no means apply to all the microscopes made by Leitz. The values must be specially determined for every instrument by the above-given method.

In conclusion, the microscopist is advised to be patient, very patient; if his preparations are unsuccessful, let him not search for the cause in the deficiency of the methods recommended—I have often tested them—but in himself; he who cannot accustom himself to conscientiously follow the written instructions,* who grasps delicate objects with his fingers, who contaminates the reagents by pouring one into the other, who leaves objects in fixing fluids exposed to the sun or allows them to become dry, has not the right to expect good results from his slovenly work.

* The periods of time given for staining, dehydrating, etc., have only an approximate value. They vary within considerable limits in accordance with the thickness of the sections, the concentration of the solutions, etc. Experience will soon teach the microscopist to determine the precise period of time.

# PART II.

## MICROSCOPIC ANATOMY AND SPECIAL TECHNIC.

The animal body consists of cells which are derived from a single cell by repeated division. At the beginning of development the cells are of similar form, all are spherical structures, and none is furnished with special characteristics that distinguish it from its companions. The cells are still *undifferentiated.* In the course of development the cells arrange themselves in flat, superposed layers, the *germ-layers.* With the separation in germ-layers and the formation of organs from these, the cells cease to resemble one another, they become *differentiated.* As a rule, the cells that have developed in the same direction are united in complexes, without definite spatial limitation, and thus form a *tissue. A tissue, therefore, is a complex of similarly differentiated cells.* We distinguish four principal tissues: 1, the *epithelial tissues;* 2, the *supporting tissues;* 3, the *muscular tissues;* 4, the *nervous tissues.* So long as these tissues are still young they consist only of similar elements, of cells; but in the course of development this condition is changed in a twofold manner. First, the cells produce special substances, which, being deposited between them, are called *intercellular substances.* By this process, however, the character of the tissue is not essentially altered. The definition of tissue given above need be only so far extended that we call a tissue a *complex of similarly differentiated cells and their derivatives.* More radical is the second change, consisting in the penetration of a tissue of one kind by other tissues. The extent of this change varies greatly in different cases. It is least marked in the case of the epithelial tissues, more so in the supporting tissues. Muscular and nervous tissues in their developed forms are mixed with other tissues to such a degree that even though among the differentiated elements muscle and nerve predominate, in the sense of the given definition they can scarcely be called tissues.* The tissues, therefore, are not equivalent among them-

* For this reason the proposition has been made to omit a division of tissues and to distinguish only elements and organs.

selves; in the lowest rank stand the epithelial tissues and the supporting tissues; both, though differing from each other in form and function, occur also in the plant-world; we can therefore class them as *vegetative tissues*. On a higher level, as well morphologically as physiologically, stand the muscular and nervous tissues, that, being found only in the animal body, are called *animal tissues*.

When different tissues unite in the formation of a body of definite internal structure and definite external form,* they constitute an *organ*.

Accordingly our task resolves itself into: 1, the study of the cells and of the tissues, and, 2, the study of the organs. The investigation of cells and of tissues is the object of *histology*. Histology is a part of general anatomy, which, because of the instrument most used in its study, is called *microscopic anatomy*. The investigation of organs, also, so far as it can be done with the aid of the microscope, is the task of microscopic anatomy.

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# I. HISTOLOGY.

(*Microscopic Anatomy of Cells and Tissues.*)

## A. CELLS.

A cell, *cellula*, is a spatially-limited structural element, which under certain conditions is able to nourish itself, to grow, and to multiply. In virtue of these properties the cell is called an *elementary organism*.

The essential parts of a cell are: 1. The *protoplasm*, or cell-body, a soft, semi-fluid substance of alkaline reaction, insoluble in water, highly distensible, that consists principally of albuminous substances, much water, and salts, and contains a special nitrogenous proteid, *plastin*. In the protoplasm small granules, *microsomes*, occur in variable quantity; when numerous they may give to the protoplasm a darker appearance. They are irregularly distributed, namely, are absent in the superficial layer, the *exoplasm*, which is somewhat denser and perhaps possesses a special function. With the aid of very high magnifying powers it is seen that protoplasm possesses structure: a reticulum, *spongioplasm*, which is

* Usually in the definition of an organ "the definite function" is included; but this does not come within the limits of a *morphologic* definition, nor is it a special peculiarity of an organ, but may be the property of a cell as well as of a tissue.

embedded in an amorphous ground-substance, *hyaloplasm* (Flemming).* 2. The *nucleus*, a clear, sharply-defined, usually vesicular body lying in the middle of the cell, that consists of several proteid substances, *chromatin*, or nuclein, *pyrenin*, or paranuclein, *linin*, "*nuclear fluid*," or matrix, and *amphipyrenin*. Chromatin and pyrenin, by their affinity for stains, are distinguished from the other three so-called achromatin substances, but differ chemically from each other. For example, on the addition of distilled water the structures composed of chromatin disappear, while those composed of pyrenin remain intact. In the simplest case (in spermatozoa), the nucleus is a compact mass of chromatin, to which the pyrenin is attached, but usually it consists of a network of fine linin-threads and coarser chromatin-cords. † The chromatin-cords are of different caliber, and at intervals exhibit isolated *enlargements* ("net-knots," karyosomes),

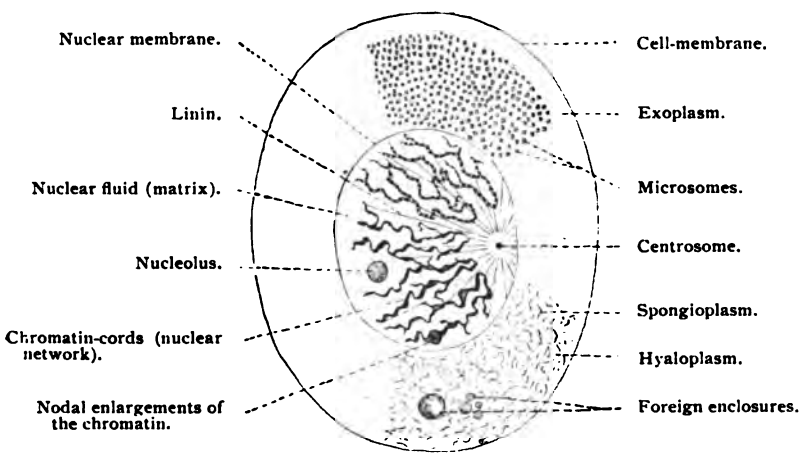


FIG. 2.—DIAGRAM OF A CELL. Microsomes and spongioplasm are only partly drawn.

that must not be confused with the nucleoli. Linin and chromatin form the *nuclear network*, the interstices of which are occupied by one or more nucleoli consisting of pyrenin and by the nuclear fluid. ‡ The nuclear

* The theories concerning the structure of protoplasm are by no means agreed. According to Fromann, Leydig, and others, protoplasm is a spongy structure, that is, it consists of a network, the meshes of which contain a fluid. According to Bütschli, the structure is froth-like, that is, it contains small spaces that do not communicate with one another. According to the much-disputed theory of Altmann, protoplasm is composed of granules (granula, bioplasts), connected by an indifferent substance, and these are the real elementary organisms.

† In suitable preparations it may be seen that the chromatin-cords are composed of rows of granules which lie in contact with threads of linin. This is shown in the upper half of the diagram (Fig. 2).

‡ Recently a special structure has been ascribed to the nuclear-sap; it is said to consist of a substance in the form of a framework, within which are a fluid and tumescent granules.

membrane, not always present, is composed of amphipyrenin; often a membrane is simulated by a thin superficial layer of chromatin. The nuclear network and the nucleoli undergo important changes according to the age of the cell. 3. The *centrosome*, a usually diminutive corpuscle within the nucleus, from which fine threads extend to the chromatin-cords and to the nuclear membrane. Because of its minuteness it can be seen only in particularly favorable objects (in the spermatocytes of Ascaris megalocephala univalens, in carcinoma cells); it becomes more distinct when it wanders from the nucleus into the protoplasm, which it does during the division of the cell. In the protoplasm the centrosome seems to be able to remain for a considerable period and there it was first discovered (Fig. 3).

Most cells contain but one nucleus; only a few have several nuclei (some wandering-cells, giant-cells, and others). Non-nucleated cells (horny cells of the epidermis, colored blood-corpuscles of mammals) originally possess nuclei, but lose them in the course of development.

FIG. 3.—CELL OF THE BONE-MARROW OF A RABBIT. × 1500. The double centrosome lies in a clear area, the attraction-sphere.

An unessential element of the cell is the *cell-membrane*, which is wanting in many cells and when present is either a transformation of the peripheral zone of the protoplasm or a secretory product of the latter; it appears as a thin, usually structureless envelope. The protoplasm of cells may contain adventitious materials, pigment, glycogen, etc., and globules of fat, of aqueous and slimy fluids. The term paranucleus has been used to designate various structures, the significance of which is not yet in each case determined. A paranucleus is often simulated by the remnants of degenerated cells that have been incorporated in a living cell. In other cases the paranucleus is confused with the centrosome.

Cells differ greatly in form. They may be: *spherical*, the typical form of all cells in the embryonal period, and in the adult, for example, resting leucocytes are spherical; *discoid*, *e. g.*, the colored blood-corpuscles; *polyhedral*, *e. g.*, the liver-cells; *cylindrical* or *columnar*, *e. g.*, the epithelium of the small intestine; *cubical*, *e. g.*, the epithelium of the capsule of the crystalline lens; *flattened* (so-called squamous epithelium), *e. g.*, the epithelial-cells of the blood-vessels; *spindle-shaped*, *e. g.*, many connective-tissue cells; *elongated into fibers*, *e. g.*, smooth muscle-fibers; and *stellate*, *e. g.*, many ganglion-cells. The form of the nucleus usually corresponds to the form of the cell. It is more or less oval in columnar,

spindle, and stellate cells; rounded in spherical and cubical cells. Lobulated, so-called polymorphous, nuclei are found in leucocytes and in giant-cells; they are a symptom of activity on the part of the cell, tending either to locomotion or change in form, or to increased metabolic energy.

The size of cells varies from forms microscopically small, 4 $\mu$* (colored blood-corpuscles), to macroscopic bodies (eggs of birds, of amphibians). The size of the nucleus corresponds in general to that of the protoplasmic body; only mature ova, despite their great dimensions, have minute nuclei.

The *vital properties* of cells will be discussed here only in so far as they can be studied by direct microscopic observation; other details must be sought in text-books of physiology. Accordingly, the phenomena of motion in cells, the reproduction of cells, and those microscopic processes which are associated with the secretory activity of cells will be considered.

FIG. 4.—LEUCOCYTES OF A FROG. × 560. Changes in form observed during ten minutes. Techn. No. 43.

The *phenomena of motion* occur in the form of ameboid † activity, of ciliary motion, and of contraction of certain fibers (muscle-fibers). The ameboid movement is the most important; it has been observed in nearly all the cells of the animal body. In well-marked cases, *e. g.*, in leucocytes, the protoplasm of the cells throws out finer or coarser processes (pseudopodia), which by dividing and flowing together produce a great variety of forms. These processes may be retracted or they may become fixed and draw the remainder of the cell-body after them, the result of which is locomotion, or the so-called "wandering" of cells. The wandering-cells play an important part in the economy of the animal body. The processes can flow around and enclose foreign particles or small cells, an incident described as the feeding of the cell. ‡ Ameboid

* A mikron, $\mu\iota\kappa\rho o\nu = \mu = 0.001$ mm.

† This movement is exhibited in its perfection by unicellular organisms named amebæ,—thence the phrase "ameboid movement."

‡ This must not be confused with the nutrition of the cell, which is effected by a series of complicated chemical processes within the cell: diosmotic currents, imbibition, molecular pressure, etc.

movements ensue very slowly ; in warm-blooded animals, only on artificial warming of the object. For ciliary motion and contraction see the Epithelial Tissues and the Muscular Tissues.

There is still another movement that is observed not only in the living but also in the dead cell. This is the so-called *molecular motion*, an oscillation of minute granules in the cell, the result of molecular currents in the fluid in which they are suspended. It may often be observed in the salivary corpuscles (see the Lymph-follicles of the Tongue).

*Reproduction and Multiplication of Cells.*—Formerly, two kinds of cell-formation were distinguished,—spontaneous generation (*generatio æquivoca*) and generation by division. According to the theory of spontaneous generation, cells originated in a suitable fluid, *cytoblastema*. This view has been utterly abandoned. Only one kind of cell-generation is now recognized ; namely, reproduction by *division* of preëxisting cells, "Omnis cellula e cellula." *

In the division of a cell, first the nucleus and then the protoplasm divides into two usually equal parts. In this process a special grouping and rearranging of the nuclear substances take place according to definite laws. This mode of division is called *indirect division*, *mitosis*,† *karyokinesis*. Its cycle is usually divided into three phases, as follows :

(1) **Prophase.**—The centrosome increases in size and migrates from the nucleus into the protoplasm. There it lies close beside the nuclear membrane, surrounded by a clear zone from which delicate threads radiate, that collectively are called *astrosphere*, or attraction-sphere. The centrosome now divides in halves, each of which is surrounded by an attraction-sphere. Then the nucleus enlarges ; the nuclear network becomes richer in chromatin and the chromatin-cords assume the form of tortuous segments, *chromosomes*, ‡ transversely dis-

* Likewise, a new nucleus can be formed only by the division of an existing nucleus. The theory of spontaneous generation of nuclei, according to which nuclei originate directly from the protoplasm and independently of existing nuclei, lacks convincing evidence.

† Μίτος = thread, because in this process threads are visible in the nucleus. There is a second mode of division, in which the nuclei divide simply by constriction, without a definite grouping of the nuclear substances. This is called *direct* or *amitotic division*. It is, however, very probable that this kind of division in vertebrates has not the significance of a *physiological* multiplication of cells, but occurs only in those cells which are on the point of disintegrating, for very often the division of the protoplasm does not follow, so that only a multiplication of nuclei takes place. This frequently happens in leucocytes, also in epithelial-cells, *e. g.*, in the superficial epithelial-cells of the bladder of young animals.

‡ These segments are also present in many resting nuclei, but are not easy to distinguish because of the many lateral branches by which they anastomose with their fellows to form a

posed to the longitudinal axis of the nucleus, the number of which is constant for each animal species. The form of these segments is usually that of a V-shaped loop. The apices or closed ends of the loops are directed toward a common center, the *polar-field*—the area in which the centrosomes are situated—their free ends toward the opposite pole of the cell. This arrangement of the segments is called the *close skein*. It is followed by a further thickening of the segments and the formation of the

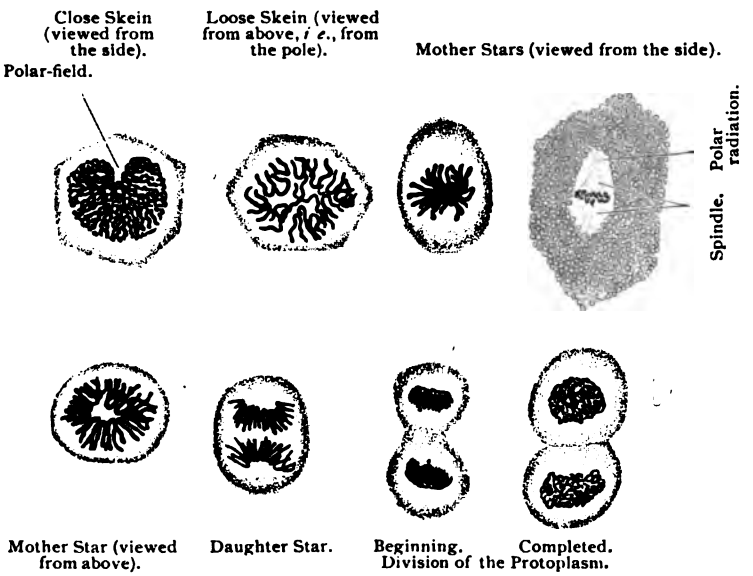


FIG. 5.—KARYOKINETIC FIGURES OBSERVED IN THE EPITHELIUM OF THE ORAL CAVITY OF A SALAMANDER. The picture in the upper right-hand corner is from a section through a dividing egg of Siredon pisciformis. Neither the centrosomes nor the first stages of the development of the spindle can be seen by this magnification. × 560. Techn. No. 1 *b*.

*loose skein*, in which the loops are less tortuous and some have their closed ends turned away from the polar-field.

Meanwhile the two centrosomes move apart and wander along the nuclear membrane, each through an arc of 90°. The interval between them is spanned by delicate fibrils, which form the "central-spindle"; to these the linin-filaments, extending from the centrosomes to the chromosomes (or chromatin-cords), become applied (see p. 57). Toward the completion of the prophase the nuclear membrane vanishes and the nucleolus becomes invisible.

(2) **Metaphase.**—The centrosomes have reached diametrically

network. When the process of division begins the lateral twigs are retracted, and consequently the segments become thicker and more conspicuous. In some nuclei the chromatin appears as a single filament, which subsequently divides into chromosomes.

opposite points * and the threads extending from them to the chromosomes, with which parts of the nuclear membrane may be associated, now appear in the figure of a spindle, the *nuclear-spindle*. At each apex of the spindle is a centrosome surrounded by an attraction-sphere, which in this stage is also known as "polar-radiation." † The chromatin-loops move to the equator of the spindle, in the future plane of division of the nucleus, and arrange themselves so that their closed ends are directed toward the axis of the spindle, their free ends toward the equator. Viewed from the apex of the spindle this grouping of the segments has the appearance of a star, *mother-star* (monaster).

During the formation of the mother-star, often earlier, in the first stages of the prophase, the chromatin-loops divide longitudinally, and each forms two "sister-loops." Division of the nucleus exactly into halves now follows, as a result of the contraction of the threads of the spindle, by which one sister-loop of a pair is drawn to one pole, the other to the opposite pole of the spindle. This process is called *metakinesis*. In this stage the nuclear segments appear in the form of two *daughter-stars* (diaster).

(3) **Anaphase.**—These figures are soon obliterated. The lateral twigs of the chromosomes reappear, anastomose with one another, and reproduce the reticulum of the resting nucleus. Meanwhile the spindle and the greater portion of the polar-radiation have become invisible, the nuclear membrane is reformed, the nucleus reabsorbs the nuclear fluid, swells and becomes spherical, and the nucleolus reappears. At the same time the hitherto quiescent protoplasm begins to divide, a furrow appearing at the equator of the cell and deepening until the separation into halves is accomplished.

In rare cases of mitotic division, especially in those of a pathologic nature, the nucleus simultaneously divides into more than two.

The duration of cell-division varies from a half hour (in man) ‡ to five hours (in amphibians).

Special modifications of cell-division are the so-called *endogenous cell-formation* and *budding*. The former occurs in those cells which are enclosed in a firm envelope (eggs, cartilage-cells), and the mode of

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* The above description of the behavior of the centrosomes does not always hold good. For example, the centrosome in Ascaris megalocephala univalens divides within the nucleus, which elongates and extrudes a centrosome at each end. During their extrusion the nuclear spindle is formed. In succeeding events the processes are identical.

† Remains of the central-spindle still lie in the axis of the nuclear-spindle.

‡ The disappearance of the mitotic figures in the human cadaver is not complete until after an elapse of forty-eight hours.

division is precisely the same as that described above, only that all the descendants of the mother-cell remain enclosed in the common capsule. Gemmation or budding indicates a kind of unequal cell-division, in which protoplasmic processes of the cell are set free by constriction and become independent cells (see bone-marrow).

The young cells always resemble in character the mother-cells. Such a case as a connective-tissue cell arising from the division of an epithelial-cell never occurs.

*The Phenomena of Secretion.*—See Secretory Activity of Epithelial Tissue.

The *length of life* of all cells is limited. The old elements disintegrate, new ones appear in their places. Formerly these phenomena were not distinguished from secretory processes, and the erroneous idea was entertained that the process of secretion terminated in the death of the cell. Dying cells are characterized by decrease in the volume of both nucleus and protoplasm. The latter often presents a notched edge or stains deeply, while the chromatin substance of the nucleus decreases or appears in the form of irregular fragments that stain uniformly. Vacuolization of the protoplasm or the nucleus is another symptom of degeneration. Dying cells in abundance may be observed in epithelia, where formerly they were often regarded as peculiar kinds of cells (cf. also Fig. 16).

The *growth* of cells preëminently concerns the protoplasm and only exceptionally takes place equally in all directions, in which case the original form of the cell is retained (*e.g.*, egg-cell); as a rule, an unequal growth occurs. As a result of unequal growth the original form is altered; the cell becomes elongated, or flattened, or branched, etc. The majority of cells are soft and susceptible to change in form from mechanical influences, as, for example, the columnar epithelial-cells in the empty bladder, which are flattened in the distended organ. Epithelial-cells of the peritoneum may through stretching acquire three times their original superficies.

*Secretory Products of Cells.*—The secreted materials are either wholly removed (as most glandular secretions) or they harden and remain on the surface of the cells. To the latter belong certain *intercellular substances*, many of which are a secretion of cells; others are produced by a transformation of the peripheral layers of the cell-protoplasm, still others, by a complete metamorphosis of the cells themselves (?). It is very difficult to decide whether individual intercellular substances were formed by one process or another; many points in this matter are still the subject of lively controversy.

The intercellular substances occur either in small amount, as structureless, soft, perhaps fluid, *cement-substance*, between epithelial-cells, connective-tissue cells, smooth muscle-fibers, etc.; or in large amounts, exceeding the mass of the cells, and are then called *matrix* or *ground-substance*. The matrix is either formless (homogeneous) or formed; in the latter case it is for the most part transformed into fibers or granules of different kinds. The remnants of formless substance found between the fibers or granules are also called cement-substance.

## TECHNIC.

No. 1.—For the study of nuclear structure and karyokinesis amphibian larvæ are most suitable. Those most readily procured are the larvæ of the water-salamander, which in the months of June and July abound in every pool. Place freshly-caught specimens, 3 to 4 cm. long, in about 100 c.c. of chromic-acetic acid (p. 22). After three hours place the larvæ in running water for eight hours, and then in 70 per cent. alcohol. At the expiration of four hours, or later, the objects are ready for further treatment.

*a. Nuclear Structure.*—With a scalpel carefully scrape the epithelium from the skin of the abdomen, with two pairs of fine forceps strip off the thin corium, stain it from one to three minutes in 5 c.c. of Hansen's hematoxylin (p. 36), and mount in damar-varnish (p. 45). Between the round glands beautiful connective-tissue cells with large nuclei may be seen. The reticulum of the protoplasm, the centrosome and attraction-sphere, and the finer structure of the nucleus can only be recognized by the employment of complicated methods and high magnification. The results obtained by ordinary methods are like that pictured in Fig. 6.

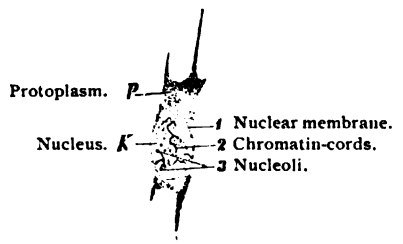



FIG. 6.—CONNECTIVE-TISSUE CELL FROM CORIUM OF TRITON TÆNIATUS. SURFACE VIEW. × 560. Only the coarser filaments of the nuclear network can be distinctly seen; with this magnification the finer filaments appear as minute dots, the nucleoli as parts of the nuclear network.

The cross-striped muscles of the tail and the membranes of smooth muscle-fibers (the latter may be readily obtained by stripping off the muscularis of the intestine) also furnish instructive slides.

*b. Karyokinesis.*—With a pair of fine scissors cut round the margin of the cornea, and strip off the same; stain and preserve as in *a*. The preparation must be placed on the slide with the convex surface of the cornea upward; in the epithelium, even with the low-power objective, many karyomitotic figures may be seen, which are recognized by their intense color. By this method the nuclear-spindle and polar-radiation, as in Fig. 5, can only be perceived (with higher magnification) in especially favorable preparations, *e. g.*, eggs of siredon and of the trout.

The delicate lamellæ suspended from the convex side of the cartilaginous gill-arch, as well as the epithelium of the floor of the oral cavity, are suitable objects. Occasionally not a single karyokinetic figure is found. Isolated figures may sometimes be observed in preparation *a*.

## B. TISSUES.

### 1. THE EPITHELIAL TISSUES.

The elements of epithelial tissue, the *epithelial-cells*, are sharply defined cells consisting of protoplasm and nucleus. A cell-membrane is frequently absent, often is represented by a condensation of the peripheral zone of the protoplasm. The majority of epithelial-cells are soft and plastic, yield readily to the pressure of neighboring cells, the result of which is great diversity of outline. In general two principal forms can be distinguished: the *flattened or squamous* and the *cylindrical or columnar* (better, prismatic). These extremes are united by numerous transitional forms.

The squamous epithelial-cells are rarely symmetrical in form, excepting the pigmented epithelium of the retina, which consists of tolerably regular hexagonal cells; generally the contour is very irregular.

FIG. 7.—ISOLATED EPITHELIAL CELLS OF RABBIT. × 560. 1. Squamous cells (mucous membrane of mouth). Techn. No. 90. 2. Columnar cells (corneal epithelium). 3. Columnar cells, with top-plate, *s* (intestinal epithelium). 4. Ciliated cells: *h*, cilia (bronchial epithelium). Techn. on p. 28.

The cylindrical epithelial-cells, *cylinder or columnar cells*, seen from the side are elongated elements, the height of which considerably exceeds the breadth; seen from above they appear hexagonal; they are therefore in reality prismatic.

Cells as high as they are broad are called cubical epithelial-cells; sometimes, pavement-cells; but since any form of epithelium viewed from the free surface may present a mosaic, the term *pavement* is not distinctive.

Many columnar cells have a sometimes homogeneous, sometimes striated border on their free upper surface (Fig. 7, 3 *s*), a cuticular formation, the so-called *top-plate*.

The striæ are the optical expression of minute rods, occasionally distinctly seen even with medium magnification (Fig. 9 *c*); they are processes of the protoplasm that penetrate the homogeneous cuticular zone, and differ greatly in length. To the same category belong the striations seen in the basal half of the cells lining the smaller ducts of the salivary glands and in the cells of some of the tubules of the kidneys; in the latter they form the so-called "brushborder," and are distinguished by their greater delicacy.

Other columnar cells are beset with delicate filamentous processes (cilia) on their free surface, that during life are in constant active vibration to and fro in a definite direction. These are called *ciliated cells.*

The specially-differentiated neuro-epithelial cells will be described in connection with the organs of special sense.

FIG. 8.—PIGMENTED EPITHELIUM OF THE RETINA OF MAN. Viewed from the surface. × 560. Techn. No. 173.

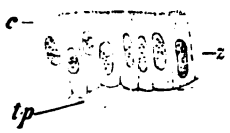



FIG. 9.—SIMPLE COLUMNAR EPITHELIUM OF SMALL INTESTINE OF MAN. × 560. *c*. Striated cuticular border. *z*. Columnar cell. *tp*. Tunica propria. Techn. like No. 102.

FIG. 10.—STRATIFIED SQUAMOUS EPITHELIUM (LARYNX OF MAN). × 240. 1. Columnar cells. 2. Prickle-cells. 3. Squamous cells. Techn. No. 122.

Continuous layers of epithelial-cells, covering outer and inner surfaces of the body, are called "epithelia." The epithelia are sometimes composed of a single stratum, sometimes of several strata, and, accordingly, the following varieties are distinguished:

1. *Simple squamous epithelium:* in the outer layer of the retina, the alveoli of the lungs, the rete vasculosum Halleri, the membranous labyrinth, the choroid plexuses and parts of the ventricles of the brain, the posterior surface of the anterior capsule of the lens, in parts of the ducts of glands, in the Malpighian corpuscle and descending limb of Henle's loop in the kidney; also in the peritoneum, the articular cavities, the tendon-sheaths, the bursæ, the blood- and lymph-vessels. The five last-mentioned epithelia are also called *endothelia*—their elements, *endothelial-cells.*

2. *Simple columnar epithelium:* in the intestinal canal and in the ducts of many glands.

3. *Simple ciliated epithelium:* in the smallest bronchi, in the uterus and oviducts, in the accessory spaces of the nasal fossæ, in the central canal of the spinal cord.

4. *Stratified squamous epithelium;* not all the elements of which are flattened cells: the lowermost stratum is composed of columnar cells; superposed on this are several strata of variously-shaped cells, mainly irregular polygonal prickle-cells, over which lie successive strata of cells that as they approach the surface become progressively thinner and flatter (Fig. 10). The stratified squamous epithelium occurs in the mouth and pharynx, in the esophagus, on the vocal cords, on the cornea, in the vagina, and in the female urethra. The epidermis also consists of a stratified pavement epithelium, but is characterized by the cornification of the cells of the superficial strata, which are transformed into horny scales without nuclei. Cornified cells are also found on the hairs and nails, but in these situations they are nucleated.

5. *Stratified columnar epithelium:* in man is found only on the conjunctiva palpebrarum, in the main excretory ducts of certain glands, and in a portion of the male urethra. The arrangement of the strata is similar to that of—

6. *Stratified ciliated epithelium:* only the most superficial cells are columnar and bear cilia; in the deepest layers the elements are mainly spherical; in the middle layers, spindle-shaped (Fig. 11). Stratified ciliated epithelium is found in the larynx, in the trachea, in the larger bronchi, in the nasal fossæ, in the upper part of the pharynx, in the Eustachian tube, and in the epididymis.

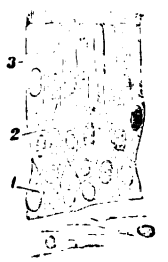
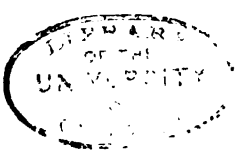



FIG. 11.—STRATIFIED CILIATED EPITHELIUM. × 560. From the respiratory nasal mucous membrane of man. 1. Oval cells. 2. Spindle-shaped cells. 3. Columnar cells. Techn. No. 193.

Between the epithelial-cells extremely narrow clefts often occur,—*intercellular spaces*,—which are occupied by a soft, perhaps fluid, *intercellular substance*.* In many epithelia—perhaps all the columnar epithelia of the mucous membranes—and in the majority of the glandular epithelia, the *intercellular spaces* are closed toward the free surface by very delicate bars of a peculiar cement-substance;

* Since, in the human skin, the intercellular spaces have been successfully injected through the lymph-vessels, it was believed that this substance is identical with ordinary lymph. This, however, is not correct, for the intercellular substance of epithelium reacts differently; it becomes black when treated with silver nitrate.



since these bars—"*terminal bars*" (*Schlussleisten*)—are connected with one another they form a "*network of terminal bars*" (*Schlussleistennetz*) in the meshes of which the ends of the epithelial-cells directed toward the free surface are inserted.

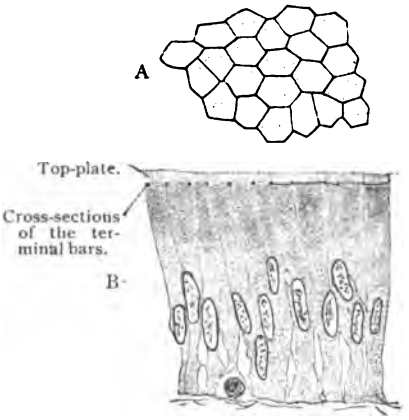



FIG. 12.—COLUMNAR EPITHELIUM OF AN INTESTINAL VILLUS OF MAN. Magnified about 600 times. Network of terminal bars: A, view of free surface; B, lateral aspect; on the left the cross-sections, on the right the lateral surfaces of the terminal bars are seen.

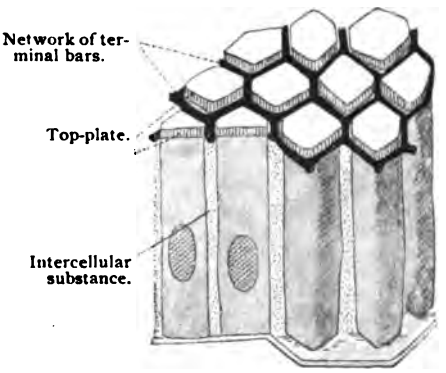



FIG. 13.—SCHEME OF THE NETWORK OF TERMINAL BARS. The two cells on the left are divided lengthwise into halves; the two on the right are drawn as complete cylinders or prisms.

The union of the epithelial-cells is effected in such a manner that either they present smooth surfaces of contact to one another, namely by the intervention of intercellular substance, or they interlock by variously-shaped processes, the latter being pressure-effects. The delicate spines and thorns visible on the surfaces of many epithelial-cells have been regarded as similar processes. But these are connecting-filaments,* which pierce the intercellular substance and establish an intimate union with neighboring epithelial-cells. Cells provided with such spikes and ridges are called *prickle-cells;* the processes are aptly designated by the appropriate name of *intercellular-bridges* (Fig. 14).

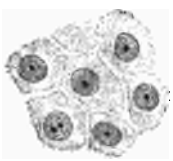


FIG. 14.—FROM A VERTICAL SECTION OF THE STRATUM GERMINATIVUM OF THE EPIDERMIS. × 560. Seven prickle-cells united by intercellular bridges. Techn. like No. 83.

They were first seen on the polygonal cells of stratified squamous

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* These filaments, that can be traced in the interior of the cells (the spongioplasm, p. 56), are the ground on which such epithelium was said to have a "fibrillar" structure,—a designation that can only lead to perplexity, because, for example, it tends to produce confusion with the fibrillar structure of connective tissue, which is something wholly different.

epithelium,* but they also occur on the cells of simple squamous and columnar epithelium, for example, of the stomach and of the intestines, but there they are extremely delicate and can be demonstrated only by the application of special methods. The length of the intercellular-bridges and the diameter of the "intercellular clefts" occurring between them vary greatly in the different forms of epithelium and in the different physiologic states of the tissue.

The epithelium has no blood- and lymph-vessels, but nerves are found in some situations, for example, in the epithelium of the skin and of many mucous membranes.

**Secretory Activity of Epithelial Tissue.**—Many epithelial-cells are capable of secreting and discharging certain substances which are not used in the growth and development of the tissue. Such cells are called *glandular cells*. The secreted substances are either used in the body (secretions) or, those of no further use, removed from the body (excre-

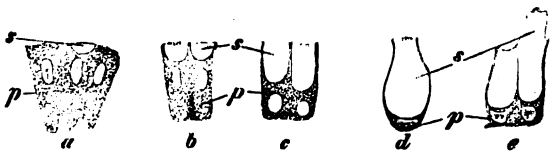


FIG. 15.—SECRETING EPITHELIAL-CELLS. From a thin section of mucous membrane of the stomach of man. × 560. *p*. Protoplasm. *s*. Secretion. *a*. Two cells empty of secretion; the cell between them shows beginning mucoid metamorphosis. *e*. The cell on the right is discharging its contents, its upper free wall having ruptured; the granular protoplasm has increased, and the nucleus has become round again. Techn. No. 102.

tions). The performance of the processes of elaboration and discharge of secretions (or excretions) is manifested by certain changes in the appearance of the form and contents of glandular cells, which indicate states of rest and activity. In many, *e. g.*, serous glandular cells, the differences (barring certain phenomena in the nucleus) are confined to decrease in volume and a dark appearance of cells empty of secretion, and to increase in volume and a clear appearance of those filled with secretion. In other gland-cells, *e. g.*, in many mucous glands, the process of secretion can be more closely traced. Granular protoplasmic contents and a usually oval, nearly centrally-situated nucleus indicate a condition of exhaustion. The elaboration of secretion begins at the free surface of the cell, that directed toward the lumen of the gland, and manifests itself by the transformation of the granular protoplasm into a clear mass (*b*, *s*), more or less sharply defined against the still unaltered

*The basal surface of the columnar cells of stratified squamous epithelium are also provided with short processes, directed toward the subjacent connective tissue, the "rivet-fibers" (*Haftfasern*), that are rendered visible only by means of complicated methods.

protoplasm (*b*, *p*). As the process of secretion progresses, more and more of the protoplasm is transformed, and the nucleus and remnant of unaltered protoplasm are pushed to the bottom of the cell; as a consequence of this compression the nucleus gradually becomes rounded or even flattened. The volume of the cell when filled with secretion is considerably increased. Finally, the cell-wall bursts at its free surface. The secretion gradually escapes, simultaneously the protoplasm is regen-

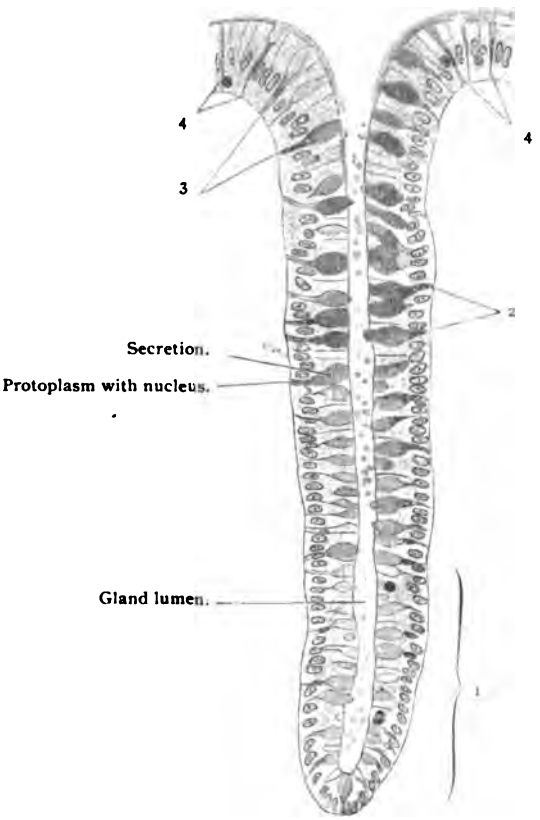


FIG. 16.—CRYPT OF LIEBERKÜHN FROM A SECTION OF THE LARGE INTESTINE OF MAN. × 165. The secretion formed in the goblet-cells is dark in color. In zone 1 the goblet-cells show the beginning of secretion. That a part of the secretion is already given off here is evident from the presence of secretion in the form of drops in the lumen of the crypt. 2. Goblet-cells with much secretion. 3. Cells containing a small amount of secretion. 4. Degenerating goblet-cells, some of which still contain remnants of secretion. Techn. on p. 29.

erated, the nucleus moves upward to its original position, and the cell, diminished in size, is restored to its previous condition and appearance. The majority of glandular cells do not degenerate in the act of secretion, but are able to repeat the process again and again. The sebaceous glands furnish an exception, for their secretion is formed by the disintegration

of cells, like the goblet-cells.* In the case of the latter the processes of elaboration and of expulsion of secretion occur simultaneously (Fig. 16); at first the secretion is produced more rapidly than it is discharged and it accumulates in the cell (Fig. 16, 2), but finally expulsion exceeds production, the cell gradually empties itself completely, and dies (Fig. 16, 4).

The glandular cells lie isolated between other epithelial-cells † or are united in groups and form *glandular tissue.*

**Supplement. The Glands.** The glands are composed almost exclusively of epithelium. Connective tissue and blood-vessels, so important from a physiologic point of view, are morphologically subordinate. Therefore, although they are organs, they may be appropriately described with the epithelial tissues.

The glands are secreting epithelial tissue, buried beneath the surface of the body, which is arranged in the form of cylindrical tubules or rounded saccules. Accordingly, two principal forms of glands are distinguished: tubular and saccular (alveolar) glands.

The *tubular* glands occur singly or united in groups; therefore they are divided as follows:

1. *Simple tubular glands,* which have the form of simple or branched tubules (Fig. 17); the latter may be called a "tubular system."

2. *Compound tubular glands,* which consist of a large, variable number of "tubular systems" (Fig. 17).

The same division is applicable to *alveolar glands.* They also may be distinguished as—

1. *Simple saccular (alveolar) glands,* which, similarly, are simple or branched saccules having an excretory duct; the latter form is termed an "alveolar system."

2. *Compound saccular (alveolar) glands,* which consist of a combination of several "alveolar systems" (Fig. 17).

Simple unbranched tubular glands: the peptic or fundus glands, the sweat-glands, and the glands of Lieberkühn.

Simple branched tubular glands: the pyloric glands, the glands of Brunner, the smallest glands of the oral cavity, the glands of the tongue, and the glands of the uterus.

Compound tubular glands: the mammary, the salivary, the lacrymal, and the

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* The testicle and ovary afford a peculiar instance, the gland-cells of which after secretion undergo further development.

† They are then called unicellular glands; they are very common among invertebrates, also occur in man as goblet-cells.

larger mucous glands,* the kidneys, the glands of Cowper, the prostate gland, the

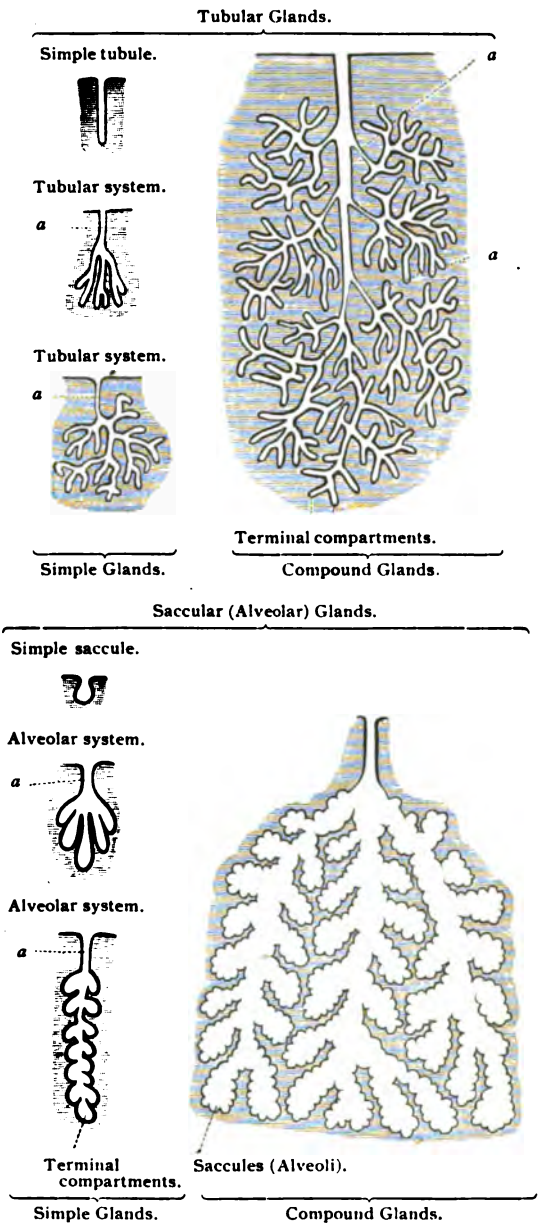


FIG. 17.—DIAGRAM OF THE DIFFERENT GLAND-FORMS. *a*. Excretory duct.

* The cross-sections of the coiled and closely-packed branching tubules of the last four glands were for a long time regarded as vesicular evaginations of the terminal ends of the tubules, and were named acini. Such evaginations (except in a few isolated parts of the sub-

thyroid gland, the testicle, and the liver. The branches in the last two anastomose and form networks, hence they are also called "reticular glands."

Simple unbranched saccular glands: the smallest sebaceous glands and the follicles of the ovary.

Simple branched saccular glands: the larger sebaceous glands and the Meibomian glands.

Compound saccular glands: the lungs.

In the majority of glands, particularly in those visible to the naked eye, a sheath is formed by the surrounding connective tissue, which sends septa into the gland and divides it into compartments of varying size, the *gland lobules.* The septa are the carriers of the larger blood-vessels and nerves. The glands may secrete throughout their entire extent, but usually only that part lying near the blind end, the *fundus*, is specialized for this purpose, while the part forming the connection with the surface serves for the conveyance of the secretion, and is called *excretory duct.*

Glands without excretory ducts are the *thyroid body* and the *ovary.* The former has an excretory duct in the embryonic period, which disappears in the course of development. The gland follicles of the ovary, in an embryonal period, also are in connection with the superficial epithelium; these connections, which might be called excretory ducts, disappear and the expulsion of the products formed in the ovary (the ova) takes place by the bursting of the follicles. The ovary is a *dehiscent* gland.

The secreting portion of all glands consists of a usually simple layer of gland-cells, which bound the lumen of the gland and are in turn surrounded by a special modification of the connective tissue, the *membrana propria* or *basement membrane** (see p. 81). On the outer side of the basement-membrane the blood-vessels are situated (Fig. 18). Hence the gland-cells are inserted between the blood-vessels and the lumen of the gland, and on the peripheral side receive from the blood-vessels (or from the neighboring lymph-vessels) the materials necessary for secre-

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lingual gland) do not really occur; the diameter of the lumen is not greater here than in other portions of the tubules. On the other hand, a thickening of the wall of terminal parts of tubules, by taller cells, is not uncommon in some tubular glands, *e.g.*, in the parotid and the pancreas. Such thickenings, however, must not be called "acini," since we understand by acinus, an evagination, a distention of the lumen. To avoid misunderstanding, the term "acinus" was dropped and that of "alveolus" selected for glands of the saccular form. The much-used term "acinous" or "racemose" has also been discarded, because the cross-sections of tubular glands also exhibit a "racemose" appearance.

* Occasionally, instead of this, the gland-tubules are embraced by stellate, nucleated cells ("basket-cells").

tion, on the other or central side yield the elaborated substances as secretion.

In many glands, for example, the mucous and the serous glands of the oval cavity, the glands of the stomach, of the duodenum, and of the pancreas, the secretion is discharged not only on the side of the cell directed toward the lumen, but on many sides. Then the secretion passes into minute canaliculi, which, simple or branched, sometimes without anastomoses, sometimes forming a network, surround the gland-cell.* These minute canaliculi, the "secretory capillaries," open individually or united in a single larger trunk into the lumen of the gland; whether they are always present or only periodically is not yet determined.

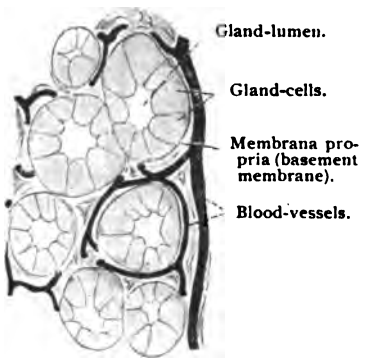


FIG. 18.—SECTION OF A MUCOUS GLAND OF THE TONGUE OF RABBIT. Blood-vessels injected. The nuclei of the gland-cells were only faintly visible in the preparation. × 180. Techn. like No. 118 b.

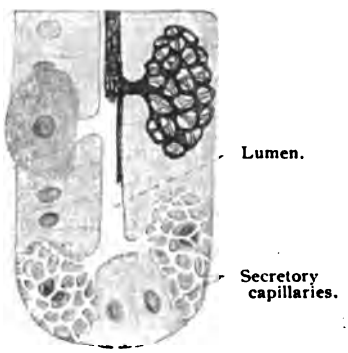


FIG. 19.—SECTION OF FUNDUS GLAND OF MOUSE. Left upper half drawn after an alcohol preparation (Techn. No. 102), right upper half after a Golgi preparation (Techn. No. 119). The entire lower portion is a diagrammatic combination of both preparations.

The microscopic appearance of the gland-cells changes with the periodic functional condition. In some glands all the cells simultaneously exhibit the same functional appearance. In other glands different functional states are encountered at the same time, even within the same tubule or alveolus. The latter is the case in many mucous glands, the cells of which have delicate walls. Tubules occur in these that contain cells in a condition of activity and of exhaustion. The loaded cells push the empty ones away from the gland-lumen; the latter then lie at the periphery of the tubule and represent in this form the so-called "demilunes of Heidenhain" or "crescents of Gianuzzi" (Fig. 20). The nuclei of many glandular cells also exhibit varying appearances corresponding to the changing functional condition; in empty cells the

* It may be that single parts of the canaliculi lie in the interior of the gland-cell.

nucleus exhibits a delicate chromatin-network and a conspicuous nucleolus (Fig. 20, I *b*), while in loaded cells the nucleolus is invisible and the chromatin-cords appear in the form of coarse fragments (Fig. 20, I *a*).

The smaller branches of the ducts of many tubular glands must be regarded as belonging to the secreting portion, since they are characterized by the specialized epithelium lining their walls and participate in the function of secretion by eliminating certain materials (salts). They are not only excretory ducts, but part of the actively-secreting portion of the gland. The difference in the structure of these branches renders their division into two parts desirable : the first portion, proceeding from the terminal compartments, is narrow and lined, sometimes with flat,

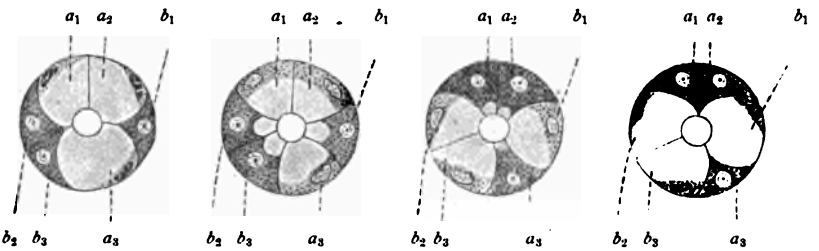


FIG. 20.—DIAGRAM OF THE ORIGIN OF THE CRESCENTS. Protoplasm shown deeply shaded, the secretion less shaded.

I. Cross-section of a tubule of a mucous gland, with six gland-cells. Three ($a_1$, $a_2$, $a_3$) are filled with secretion, and have pressed the three cells ($b_1$, $b_2$, $b_3$) empty of secretion away from the gland-lumen. Comp. Fig. 168.

II. Same section somewhat later. The cells $a_1$, $a_2$, $a_3$ have discharged a part of their secretion and become smaller. The cells $b_1$, $b_2$, $b_3$ again extend to the lumen and begin to secrete.

III. Same section still later. The cells $a_1$, $a_2$, $a_3$ have discharged the bulk of their secretion and become still smaller. In the cells $b_1$, $b_2$, $b_3$ the secretion has accumulated to such an extent that they are the larger and compress their neighbors, $a_1$, $a_2$, $a_3$.

IV. Same section still later. The cells $a_1$, $a_2$, $a_3$ are now entirely empty and pressed away from the gland-lumen by $b_1$, $b_2$, $b_3$, now full of secretion.

In I the cells *b*, in IV the cells *a*, are the crescents.

sometimes with cubical, cells ; it is called the *intercalated or intermediate tubule*. The adjoining portion is wider and clothed with tall columnar cells, the bases of which show distinct longitudinal striation ; it is called the *intralobular* or *secretory* (*salivary or mucous*) *tube*. The relative length of the intercalated tubules and the intralobular tubes varies greatly in the different glands.

The *excretory ducts* consist of a simple or stratified columnar epithelium lining a wall of connective tissue mingled with elastic fibers.

The most complex glands consist of the following sections : (1) The excretory duct, which divides into (2) the secretory tubes, which lead into (3) the intercalated tubules, which pass into (4) the terminal compartments, which, finally, take up (5) the secretory capillaries.

## TECHNIC.

No. 2.—To obtain living ciliated-cells, kill a frog (p. 27), place it on its back, and with scissors cut off the lower jaw, so that the roof of the cavity of the mouth is exposed. From the mucosa of the roof cut out a small strip about 5 mm. long, place it on the slide in a drop of salt solution, and apply a cover-glass. Examine with the high power and search the edges of the preparation. At first the movement of the cilia is very lively, so that the observer cannot see the individual cilia; the entire ciliated border waves; the motion has been compared to that of a corn-field swayed by the wind. After a few moments the rapidity of the movement diminishes and the cilia can be plainly seen. If the movement ceases, it can be restored by the application of a drop of concentrated potash solution (p. 22); the effect is transient, so that the eye of the observer must not be removed from the ocular while the fluid passes under the cover-glass. The addition of water soon suspends the movement.

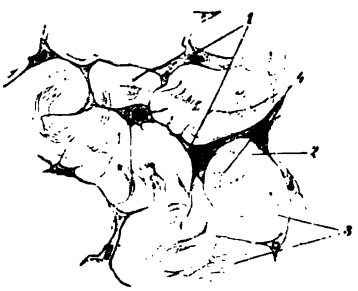


FIG. 21.—FROM A CROSS-SECTION OF THE UMBILICAL CORD OF A FOUR MONTHS' HUMAN EMBRYO. × 240. 1. Cells. 2. Intercellular substance. 3. Connective-tissue bundles mostly in oblique section, at 4 in true cross-section. Techn. No. 3.

FIG. 22.—CONNECTIVE-TISSUE BUNDLES OF VARIOUS THICKNESSES FROM THE INTERMUSCULAR CONNECTIVE TISSUE OF MAN. × 240. Techn. No. 4.

## II. THE SUPPORTING TISSUES.

While in the epithelial tissues the cells constitute the principal mass, in the supporting tissues the *intercellular substance* (ground-substance, matrix) is conspicuously developed and variously differentiated. The predominance of the intercellular substance, which also functionally plays the more important part, is characteristic of the group of supporting tissues. According to the nature of the intercellular substance they are divided into: (1) connective tissue; (2) cartilage; (3) bone.

**1. Connective Tissue.**—The matrix or intercellular substance of connective tissue is more or less soft; the cells are few in number.

Several varieties are distinguished: (*a*) mucous connective tissue, (*b*) fibrillar connective tissue, and (*c*) reticular connective tissue.

(*a*) *Mucous connective tissue* consists of round or stellate branched cells and a large quantity of undifferentiated, muciferous intercellular substance containing a few minute bundles of fine fibrils. In the higher animals it is found only in the umbilical cord of very young embryos, but it is widely distributed in many lower animals.

(*b*) *Fibrillar connective tissue* consists of abundant intercellular substance and of cells.

The intercellular substance is composed of *fibrils*, that, according to one view, are a metamorphosis of the matrix; according to another, a

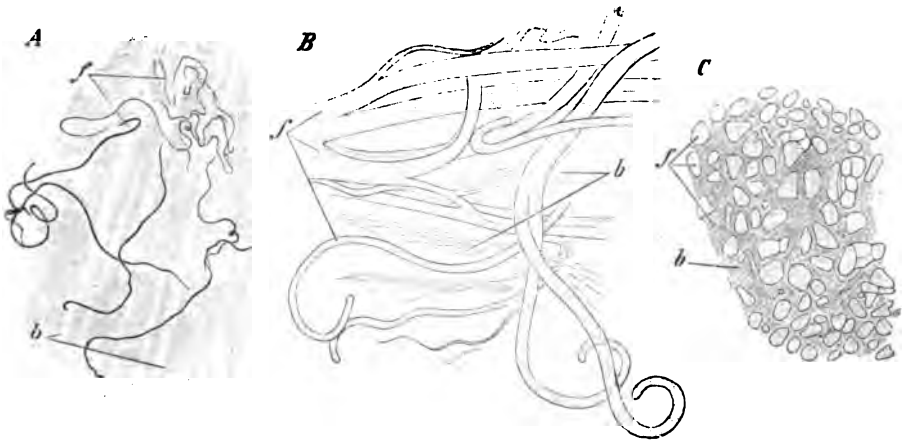


FIG. 23.—ELASTIC FIBERS. × 560. *A*. Fine elastic fibers: *f*, from intermuscular connective tissue of man; *b*, connective-tissue bundles swelled by treatment with acetic acid. Techn. No. 11. *B*. Very thick elastic fibers: *f*, from ligamentum nuchæ of ox; *b*, connective-tissue bundles. Techn. No. 12. *C*. From a cross-section of the ligamentum nuchæ of ox; *f*, elastic fibers; *b*, connective-tissue bundles. Techn. No. 13.

direct transformation-product of the cell-substance. They are exquisitely fine filaments (0.6 $\mu$), which are united by a small quantity of homogeneous cement-substance into bundles varying in thickness, the *connective-tissue bundles*. These bundles are soft, flexible, slightly extensible, and characterized by their pale contour, their longitudinal striation, their wavy course, and by their chemical properties. On treatment with picric acid they separate into their fibrils, swell on the addition of dilute acids, *e. g.*, acetic acid, and become transparent, are destroyed by alkaline fluids, and on boiling yield *glutin*.

The matrix of fibrillar connective tissue always contains *elastic fibers*, but in different quantities (Fig. 23). In contrast to the connective-tissue bundles they are characterized by their sharp, dark outlines, their strong

refractive power, and their conspicuous resistance to acids and alkalies. The elastic fibers vary from immeasurably fine to 11 μ, and usually occur in the form of finer or coarser networks, the meshes of which are sometimes narrow, sometimes large.

Narrow-meshed networks composed of thick elastic fibers form the transition to elastic membranes, which are either homogeneous or finely striated and perforated with apertures of different sizes (hence the name fenestrated membranes), and probably are produced by the merging of broad elastic fibers (Fig. 24).

When the quantity of elastic fibers predominates over the number of connective-tissue bundles, the tissue is spoken of as *elastic tissue*.

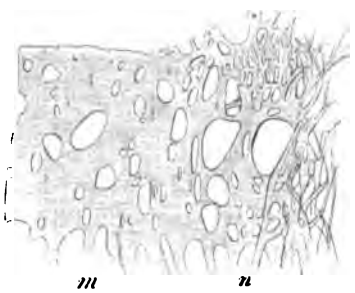


FIG. 24.—Network (*n*) of thick elastic fibers, on the left passing into a fenestrated membrane, *m*. From the endocardium of man. × 560. Techn. No. 14.

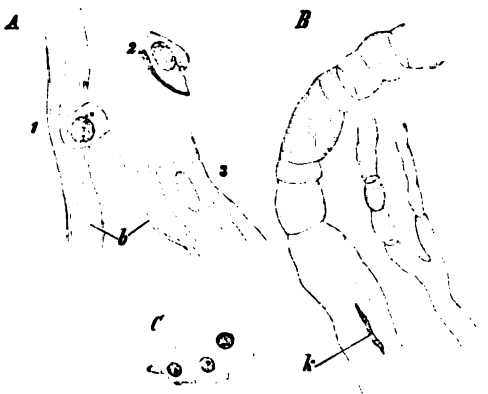


FIG. 25.—*A*. Connective-tissue cells from intermuscular connective tissue. × 560. 1. Flat cell lying partly on a connective-tissue bundle; 2, folded cell; 3, cell of which the protoplasm is not visible; *b*, connective-tissue bundles. Techn. No. 5. *B*. Connective-tissue bundles with encircling fibers; *k*, nucleus. Techn. No. 8. *C*. Plasma-cells from the eyelid of a child. Techn. No. 184.

The elastic fibers are derived neither from cells nor from nuclei, but are transformations of the matrix, perhaps of the existing connective-tissue bundles. In the beginning of their development they are thin, but become thicker with advancing growth.

The connective-tissue cells are irregularly polygonal or stellate, much flattened, variously bent or folded (Fig. 25, *A*). The flattening and bending are explained by the adaptation of the cells to the narrow spaces occurring between the connective-tissue bundles. Not infrequently the flattened cells form complete sheaths about the connective-tissue bundles. If such a bundle be treated with acetic acid, it swells and bursts the ensheathing cells, of which annular or other-shaped fragments remain and constrict the swelled bundle. Formerly these

remnants of cells were considered fibers, and were called "encircling fibers" (Fig. 25, *B*). Other connective-tissue cells are spherical, rich in protoplasm, coarsely granular, and relatively of large size; they are termed *plasma-cells* and are found principally in the neighborhood of small blood-vessels (Fig. 25, *C*). Others again, the *mast-cells*, are characterized by the affinity of their protoplasm for certain anilin dyes (*e. g.*, dahlia), but do not, as their name may suggest, stand in any demonstrable relation to the processes of nutrition. (They are also known as *granule-cells*.) The protoplasmic body of the connective-tissue cells encloses a nucleus and often contains pigment-granules; in the latter case they become *pigment-cells*, that in man are found only in certain parts of the skin and of the eye, but in the lower animals are very common. Connective-tissue cells may contain fat-globules, that, when they are very large, coalesce and give a spherical form to the cell, which is then designated a *fat-cell* (Fig. 26). In such cells the protoplasm occupies only a nar-

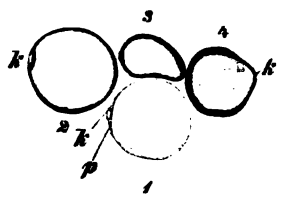


FIG. 26.—FAT-CELLS FROM THE AXILLA OF MAN. × 240. 1. The equator of the cell in focus; 2, objective somewhat elevated; 3, 4, forms changed by pressure; *p*, traces of protoplasm in the vicinity of the flat nucleus, *k*. Techn. No. 9.

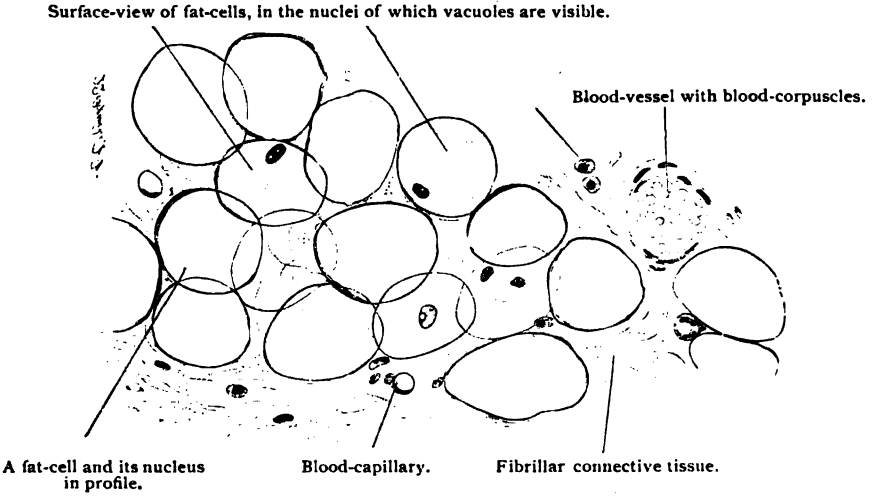


FIG. 27.—ADIPOSE TISSUE FROM THE HUMAN SCALP. × 240 (about). Techn. No. 10.

row peripheral zone, in which lies the extremely flattened nucleus, that in well-developed, but not in atrophic, fat-cells invariably contains one or more sharply-circumscribed vacuoles. These finally

pass into the interior of the fat-cell, whereupon new vacuoles form within the nucleus. The protoplasmic zone is often so thin as to be invisible. Aggregations of fat-cells are abundantly supplied with blood-vessels, lymph-vessels, and nerves, and form what is called *adipose tissue*, which bears a very important physiologic relation to metabolism.

In cases of extreme emaciation the fat in fat-cells is reduced to a few small globules. In place of the fat which has disappeared there is a pale protoplasm mixed with a mucoid fluid; the cell is no longer spherical, but has become flattened. Such cells are named *serous fat-cells* (Fig. 28). In many fat-cells after death spherical masses of needle-shaped crystals appear, the so-called *margarin crystals*.

Finally, smaller irregularly-spherical cells are found in connective tissue that are not connective-tissue elements, but leucocytes that have passed out of the blood-vessels. They are described as *wandering cells*, in distinction to those of the connective tissue, which are designated as *fixed cells;* a classification that cannot be rigidly carried out, since in some conditions (mainly pathologic) the fixed connective-tissue cells, also epithelial and glandular cells, can migrate, and it is therefore better to term the latter "histogenetic," the leucocytes "hematogenetic" wandering cells. It is self-evident that such wandering cells cannot be included in the same category with the leucocytes.

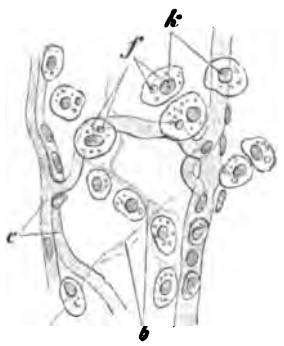


FIG. 28.—SEROUS FAT-CELLS FROM THE AXILLA OF AN EXTREMELY EMACIATED INDIVIDUAL. × 240. *k*. Nucleus; *f*, oil-droplets. *c*, Blood-capillaries; *b*, connective-tissue bundles. Techn. No. 9.

The number and distribution of the different kinds of cells are subject to considerable fluctuation.

The different elements of fibrous connective tissue are united either without exact arrangement, as in areolar tissue, or are regularly disposed in definite structures. Areolar tissue is distinguished by its loosely-connected bundles of fibers interlacing in every direction; it occurs between neighboring organs and serves to connect them and to fill in the interspaces. For this reason it is also called "interstitial" tissue. The cells of areolar tissue not infrequently contain fat. The fibrous connective tissue characterized by closer connection and regular arrangement of the bundles comprises the corium, the serous membranes, the periosteum, the perichondrium, the tendons, the fasciæ, the ligaments;

the compact sheaths of the central nervous system, of the blood-vessels, of the eye, and of many glands.

The fibrous connective tissue in immediate contact with epithelium is usually modified, forming a structureless membrane called *basement membrane* or *membrana propria*, also *hyaloid membrane*. The membrana propria of many glands, for example, the salivary glands, consists of flattened, often stellate cells, which, basket-like, surround the gland-tubules.

(*c*) *Reticular Connective Tissue.*—The views in regard to the structure of reticular connective tissue are divided. According to an opinion

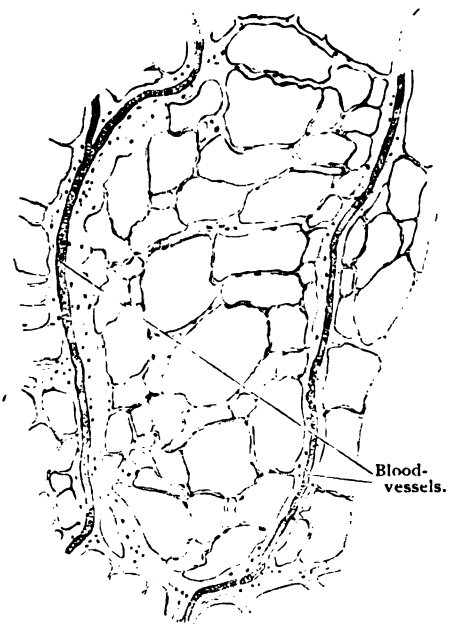


FIG. 29.—A PIECE OF THE GREATER OMENTUM OF MAN. × 60. Techn. No. 15.

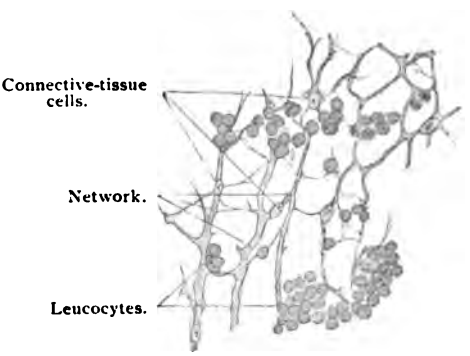


FIG. 30.—RETICULAR CONNECTIVE TISSUE. From a shaken section of a human lymph-gland. × 560. Techn. No. 50.

formerly widely entertained, it consists of a delicate network of anastomosing stellate cells. To this may be traced the name "cytogenous," that is, formed of cells, and accordingly mucous tissue may be termed cytogenous tissue. There is no doubt that such networks occur in lower animals and in embryonic stages of higher animals. But in the higher vertebrates the relations are changed; here the network consists of slender bundles of fibrillar connective tissue, upon which lie flattened, nucleated cells (Fig. 30). By means of complicated methods the outlines of the cells on the fibers can be demonstrated. In fibrillar connective tissue the

cells almost without exception lie upon the fibers. Finally, the fact that even in the adult fibrillar connective tissue may change into reticular tissue can be comprehended only on the assumption that the latter is a network of delicate fiber-bundles. Therefore reticular connective tissue really is only a variety of fibrillar connective tissue. The meshes of reticular connective tissue are usually crowded with leucocytes. It principally occurs in lymph-glands and is then called *adenoid* tissue.

**2. Cartilage.**—The matrix of cartilage is firm, elastic, easily cut, and milk-white or yellowish in color. The cells present little that is characteristic in form; usually they are spherical or, from being flattened on one side, somewhat angular. They lie in the spaces or *lacunæ* of the

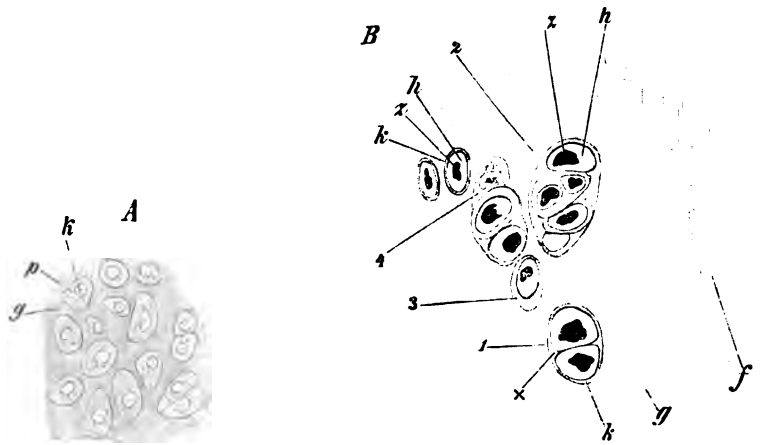


FIG. 31.—HYALINE CARTILAGE. × 240. *A*. Surface view of the ensiform process of frog, fresh; *p*, protoplasm of cartilage-cell, which entirely fills the lacuna; *k*, nucleus; *g*, hyaline matrix. Techn. No. 16. *B*. Portion of cross-section of human rib-cartilage several days after death, examined in water: the protoplasm, *z*, of the cartilage-cells has withdrawn from the walls of the lacunæ, *h*; the nuclei are invisible. 1. Two cells within one capsule, *k*; *x*, a developing partition. 2. Five cartilage-cells within one capsule; the lowest cell has fallen out, so that only the empty cavity is seen. 3. Capsule cut obliquely, and apparently thicker on one side. 4. Capsule not cut, but showing the cell within. *g*. Hyaline matrix transformed into rigid fibers, *f*. Techn. No. 17.

matrix, which they completely fill. Whether, as in bone, the matrix is penetrated by a system of minute channels communicating with and connecting the lacunæ is extremely doubtful. Many observations in which such channels apparently were perceived have been acknowledged as erroneous; the supposed channels were a result of shrinkage, and can be produced by treating cartilage with absolute alcohol or ether. Not infrequently the matrix immediately surrounding the lacunæ is specialized, and forms a strongly refractive, occasionally concentrically-striated *capsule*. The matrix is produced by the cartilage-cells; it originates in secretions that subsequently fuse into a homogeneous mass. The parts lying nearest to the cells, immediately adjoining the capsule, are the

youngest; they do not always persist, but during the process of cell-division are resorbed. Thus the ground-substance is subjected to many changes. It may be free from fibrous admixture or it may be penetrated by elastic fibers or by connective-tissue bundles. Accordingly three varieties are distinguished: (*a*) *hyaline cartilage*, (*b*) *elastic cartilage*, (*c*) *fibro-cartilage*.

(*a*) *Hyaline cartilage* is of a faint bluish, pearly color. It occurs as the cartilages of the respiratory organs and of the nose, as the costal and the articular cartilages, also in the synchondroses, and in the embryo in many situations where it is later replaced by bone. It is characterized by the homogeneity of its matrix, which in the ordinary methods of investigation appears amorphous throughout, but after special processes, *e. g.*, artificial digestion, falls apart into bundles of fibers. Further evidence in confirmation of its fibrillar structure is afforded by its appear-

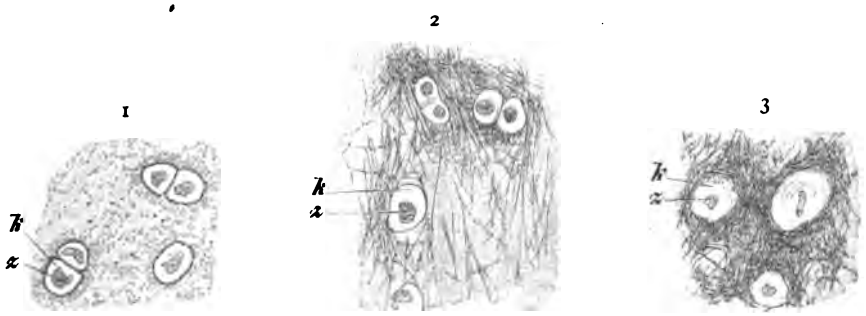


FIG. 32.—ELASTIC CARTILAGE. × 240. 1. Portion of section of the vocal process (anterior angle) of arytenoid cartilage of a woman thirty years old; the elastic substance in the form of granules. 2 and 3. Portions of sections of the epiglottis of a woman sixty years old; a fine network of elastic fibers in 2, a coarser network in 3. *z*. Cartilage-cell, nucleus not visible; *k*, capsule. Techn. No. 18.

ance when examined in polarized light. It is very firm, very flexible, and on boiling yields *chondrin*.

In certain cases the matrix may undergo peculiar modifications. In the thyroid and costal cartilages it is transformed patchwise into rigid fibers, that impart an asbestos-like luster, perceptible on macroscopic inspection. In advanced age deposition of calcareous salts may take place in the hyaline matrix, in the beginning appearing in the form of minute granules, subsequently as complete husks surrounding and enclosing the cells. In the cartilages of the larynx this may occur as early as the twentieth year.

The cells of hyaline cartilage frequently occur in groups or nests, an arrangement explained by the conditions and processes of growth. Two cells may lie in one lacuna and be enclosed within the same capsule (Fig. 31, *B* 1); they are the descendants of one cell which has under-

gone division by the indirect mode; in some cases, a thin partition of hyaline substance may be seen between two such cells. In other cases the septum does not develop immediately, and the process of cell-division may be repeated until groups of four, eight, and even more cells may be enclosed within one capsule (Fig. 31, *B*, 2). Such phenomena were supposed to establish a special theory of cell-division, the so-called endogenous cell-formation. Not infrequently the cartilage-cells in adults contain oil-globules.

(*b*) *Elastic cartilage* has a faint yellowish color. It occurs as the cartilages of the external ear, of the epiglottis, of Wrisberg and Santorini, and of the vocal process (anterior angle) of the arytenoid cartilages. It presents the same structural peculiarities as hyaline cartilage, but is distinguished by the networks of finer or coarser elastic fibers that penetrate the matrix. The elastic fibers do not arise directly from the cartilage-cells, but by a transformation of the matrix, and appear in the vicinity of the former as minute granules, that later are disposed in linear rows and fuse into fibers. This phenomenon, according to an opposite view, is regarded as an indication of post-mortem disintegration of the elastic fibers.

FIG. 33.—FROM A HORIZONTAL SECTION OF THE INTERVERTEBRAL DISC OF MAN. *g*. Fibrillar connective tissue; *z*, cartilage-cell (nucleus invisible); *k*, capsule surrounded by calcareous granules. × 240. Techn. No. 19.

(*c*) *Fibro-cartilage* is found in the intervertebral discs, the pubic symphysis, the inferior maxillary and the sterno-clavicular articulations. The matrix contains an abundance of fibrous connective tissue in loose bundles extending in every direction (Fig. 33, *g*). The cartilage-cells are few in number, have thick capsules, and occur in small groups or rows at comparatively wide intervals.

3. **Bone.**—The matrix of bone, osseous tissue, is distinguished by its hardness, solidity, and elasticity, properties due to an intimate blending of organic and inorganic substances. This union is of such a nature that either part may be removed without destroying the structure of the tissue. On treatment with acids, the inorganic substances are withdrawn; the bone is decalcified, is rendered flexible, and is easily cut, like cartilage. The organic substances may be removed by cautious heating; the bone is then said to be calcined. Similarly, fossil bones are deprived of the organic substances through the prolonged action

of moisture. The matrix or ground-substance is composed of calcium salts, chiefly basic calcium phosphate, and of collagenous fibrils, that are united by a small amount of cement-substance in finer or coarser bundles; accordingly, a *fine-textured*, or lamellar, and a *coarse-textured*, or plexiform matrix are distinguished. It appears homogeneous or faintly striated and contains numerous spindle-shaped spaces 15 to 27 $\mu$ in length, the *lacunæ*, which communicate with one another through numerous branched minute canals, the *canaliculi*. In this way a system of canaliculi that penetrates the entire matrix is established. Within the lacunæ, sometimes improperly called "bone-cells," lie nucleated, flattened, oval bodies, the real *bone-cells*. It is doubtful whether in the adult the bone-cells are connected by means of processes extending through the canaliculi, although such connection is readily observed in developing bone.*

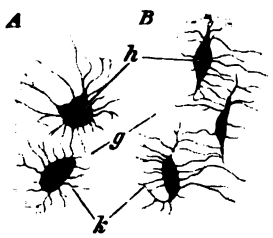


FIG. 34.—FROM A GROUND SECTION OF DRIED BONE OF ADULT MAN; *h*, lacunæ; *k*, canaliculi; *g*, bone-matrix. *A*. Seen from the surface. *B*. Seen from the side. × 560. Techn. No. 56.

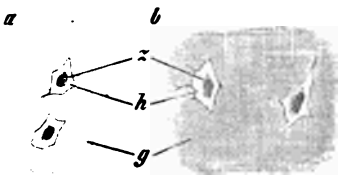


FIG. 35.—FROM SECTIONS, *a*, OF THE HUMERUS OF A FOUR MONTHS' HUMAN EMBRYO; *b*, of the middle turbinal bone of adult man; *z*, bone-cells lying in the lacunæ, *h*; the canaliculi are only partially visible; *g*, matrix. × 560. Techn. No. 62.

Fibrous connective tissue and cartilage may be converted directly into osseous tissue by calcification of the matrix; the connective-tissue cells or cartilage-cells then become bone-cells. However, this process is of comparatively rare occurrence. Usually the formation of osseous tissue takes place in such a way that the ground-substance of the connective tissue or of the cartilage calcifies during embryonic life. Around the trabeculæ of the calcified matrix numerous young, still indifferent, connective-tissue cells then arrange themselves, which produce the at first soft, then calcified, ground-substance of bone. These cells are

* The skeleton of the adult is principally formed of the fine-textured matrix, which is characterized by the arrangement of the fiber-bundles in lamellæ and contains elastic fibers. The coarse-textured matrix occurs in the fetus in periosteal and intermembranous bone, and is found in the adult along sutures and at the point of insertion of tendons; it always contains uncalcified connective-tissue bundles, the so-called Sharpey's fibers, which also are found in the circumferential and interstitial lamellæ of fine-textured bone, the remains of the primary or periosteal bone.

called *osteoblasts*. At first they lie upon the osseous matrix they have formed, later they come to lie within it, and gradually become transformed into stellate bone-cells.

*Dentine* is a modification of bone, from which it is distinguished by its developmental history; the formative cells, the *odontoblasts*, are not enclosed within the matrix, but penetrate the latter with their processes. Further details will be found in connection with the structure of teeth.

*Blood-vessels, Lymphatics, and Nerves.*—The supporting tissues are, in general, poorly supplied with blood-vessels, lymph-vessels, and nerves. An exception occurs in adipose tissue, which has a rich vascular supply. But connective tissue plays a very important part as a conveying appa-

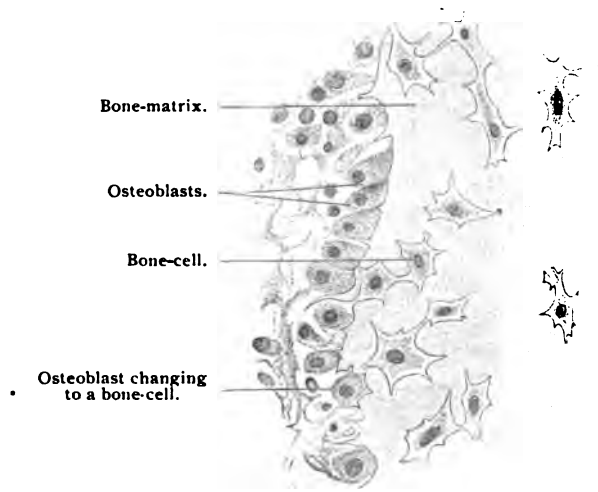


FIG. 36.—PORTION OF CROSS-SECTION OF THE DIAPHYSIS OF THE HUMERUS OF A FOUR MONTHS' HUMAN EMBRYO. × 560. Techn. No. 62.

ratus in the transference of nutritive fluids—*tissue-juices, lymph*—from the blood-vessels to the tissues. When the matrix is soft, as in mucous tissue, the lymph permeates the entire substance; when on the other hand it is denser, the lymph circulates in a system of intercommunicating channels, a *juice-canal-system*, formed by the cell-spaces—*lymph-spaces*—and the minute canals connecting them—*lymph-capillaries*. This is the case in bone and the more compact connective tissues. Whether the tissue-juice is diffused throughout the matrix of hyaline cartilage or conveyed in definite channels is still undetermined. The intercellular substance of epithelium is in direct connection with the lymph-capillaries of the subjacent connective tissue, and may be regarded as being similarly permeated by the lymph.

## TECHNIC.

No. 3.—*Mucous Connective Tissue.*—Place the umbilical cord of a three or four months' human embryo (or pig embryo from three to six cm. long) in 100 c.c. of Müller's fluid (p. 21) for three or four weeks; harden in 30 c.c. of gradually strengthened alcohols (p. 33). The cord will still be very soft; in order to obtain good sections it must be embedded in liver, and in cutting must be somewhat compressed with the fingers. The section may be stained in picrocarmine (twelve hours) or in hematoxylin (five minutes), and should be examined in a drop of distilled water. In glycerol and in damar-varnish the delicate processes of the cells and the bundles of connective tissue are invisible. In the vicinity of the blood-vessels the network of cells is less fine; therefore a field remote from the blood-vessels should be selected for study. The older the embryo, the greater is the number of the connective-tissue bundles. Mount in diluted glycerol (p. 22).

No. 4.—*Fibrous Connective Tissue; Connective-tissue Bundles.*—Prepare small strips, one or two cm. long, of intermuscular connective tissue, for example, of the thin septum between the serratus and intercostal muscles; place a small piece on a dry slide and quickly spread it out with teasing needles (see "half-drying method" No. 29 *a*, p. 106), add a drop of salt solution and apply a cover-glass. The bundles of connective tissue appear wavy and pale; with a little practice the sharply-contoured, highly-refracting elastic fibers may be distinguished and also, in favorable situations, the nuclei of the connective-tissue cells.

No. 5.—The *cells of fibrous connective tissue* may be rendered visible by the addition of a drop of picrocarmine to preparation No. 4, under the cover-glass (p. 48). In most cases only the red nucleus can be perceived, especially when the cell lies wholly upon the fibrous bundles. In rare cases the pale yellow, variously-shaped body of the cell can be seen (Fig. 25, *A*, 1, 2, 3).

No. 6.—*Mast-cells* (granule-cells).—Fix small pieces, 1 or 2 cm. square, of mucous membrane (of the mouth, pharynx, or intestine) in ninety-five per cent. alcohol (p. 30). In from three to eight days cut thin sections and stain them in 10 c.c. of alum-carmine dahlia for twenty-four hours (p. 25). Transfer them to 10 c.c. of absolute alcohol for twenty-four hours, which must be renewed once or twice during this time. Mount in damar (p. 45). The protoplasm of the mast-cells exhibits granules stained an intense blue.

No. 7.—*Fibrillæ.*—Place a piece of tendon about 2 cm. long in a saturated aqueous solution of picric acid. On the following day, with two pairs of forceps, pull the tendon apart along its length, take from the interior a bundle about 5 mm. long, and tease the same on a dry slide (*cf.* No. 29 *a*, p. 106); add a drop of distilled water, apply a cover-glass,

and examine with the high-power objective. The ultimate fibrillæ appear as exceedingly fine, silky filaments.

No. 8.—"*Encircling Fibers.*"—With the scissors cut out a piece about one cm. square of the connective tissue within the arterial circle of Willis, wash it in a watch-glass in salt solution, with needles spread it out in a drop of the same solution on a slide, and cover. With the low power, in addition to numerous delicate blood-vessels and ordinary bundles of fibrous tissue, sharply-contoured, refracting bundles, in distinct contrast to the remaining connective tissue, will be found, which, on the use of the high power and a diaphragm of narrow aperture, show that they, likewise, consist of fibrillar connective tissue. Place such a bundle in the field and treat it with a drop of acetic acid, under the cover-glass (p. 48). So soon as the acid reaches the bundle, it swells, the fibrillation vanishes, and instead elongated nuclei appear. The swelling is not uniform; at irregular intervals the bundle is constricted. With dim illumination the "fibers" (cell-remnants) producing the constrictions may be seen (Fig. 25, *B*).

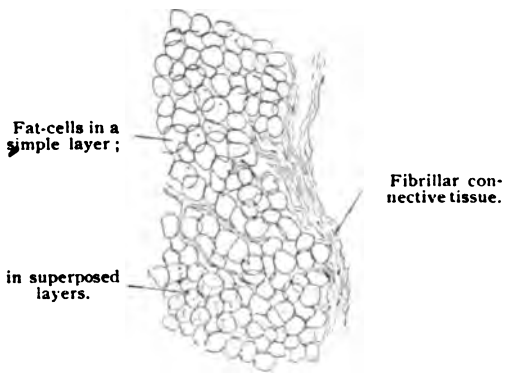


FIG. 37.—ADIPOSE TISSUE FROM A SECTION OF HUMAN SCALP. × 50. Techn. No. 161.

No. 9.—*Fat-cells.*—Take a small piece of the reddish-yellow, gelatinous fat from the axilla of an emaciated individual; *rapidly* spread out a piece the size of a split pea in the *thinnest possible* layer on a dry slide, *immediately* add a drop of salt solution and apply a cover-glass. In thin places atrophic fat-cells, like those shown in Fig. 28, will be seen. This preparation may be stained under the cover-glass with picrocarmine (p. 48) and preserved in diluted glycerol. Ordinary (normal) fat-cells, taken from any part of the body, are likewise to be examined in salt solution. The spherical cells should be studied with change of focus (*cf.* Fig. 26).

No. 10.—*Adipose tissue* may be seen in sections of many preparations fixed by any of the usual methods—above all of the skin (*cf.* Fig. 238 and 243). The oily contents are withdrawn by the treatment with

alcohol and then the clusters of empty cell-envelopes present a picture that the beginner often finds difficulty in understanding.

No. 11.—*Fine elastic fibers* may be readily obtained by treating preparation No. 4, under the cover-glass, with a few drops of acetic acid. The connective-tissue bundles swell and become transparent; the elastic fibers, on the contrary, remain unaltered, and stand out sharply contoured (Fig. 23, *A*).

No. 12.—*Thicker elastic fibers* may be obtained by teasing in a drop of salt solution a slender piece, about 5 mm. long, of the fresh ligamentum nuchæ of an ox (Fig. 23, *B*). The piece should not be taken from the loose, enveloping tissue, but from the tough, yellowish, fibrous portion. The preparation may be stained in picrocarmine and mounted in glycerol.

No. 13.—*Cross-sections of thick elastic fibers* may be obtained by drying a piece (10 cm. long and from 1 to 2 cm. thick) of the ligamentum nuchæ (it will be ready to use in four or six days) and treating it like No. 64.

No. 14.—*Fenestrated Membranes.*—Take a small piece (about 5 mm. square) of endocardium, place it in a drop of water on a slide, and add, under the cover-glass, 1 or 2 drops of potash-lye. Examine the edges of the preparation (Fig. 24).

Good specimens may also be obtained from the basilar artery; place a piece of the artery cut open lengthwise in 10 c.c. of concentrated potash solution. After six hours take a small piece, about 1 cm. long, and separate the lamellæ in a drop of water on a slide; this is easily done by scraping with a scalpel. Cover and examine with the high power. The small apertures in the membrane have the appearance of shining nuclei.

With the low power the membrane is recognized by its dark outlines. To preserve, wash it well in 10 c.c. of water (five minutes), stain it in 3 c.c. of congo-red for from twelve to twenty hours (p. 25), and mount in damar.

No. 15.—A *network of connective-tissue bundles* may be obtained by spreading out a little piece of fresh human omentum in a few drops of picrocarmine. It may be preserved in diluted, non-acidulated glycerol (p. 22). Pieces of the omentum fixed in absolute alcohol and stained with hematoxylin and eosin (p. 37) may be mounted in damar-varnish (p. 45). (Fig. 29, p. 81.)

No. 16.—*Hyaline Cartilage.*—Cut off the extremely thin episternum of the frog, place it on a dry slide, cover it with a cover-glass, and examine at once with the high power. The cartilage-cells completely fill the lacunæ (Fig. 31, *A*). For prolonged study, add a drop of saline solution.

No. 17.—*Hyaline Costal Cartilage.*—Without any previous preparation thin sections of costal cartilage may be cut with a dry razor and examined in a drop of water. Search for one of the glossy areas con-

taining rigid fibers (Fig. 31 *B*). The preparation may be preserved by adding a few drops of dilute glycerol.

Fresh cartilage does not readily stain. The tissue must be first placed in Kleinenberg's picrosulphuric-acid mixture or in Müller's fluid, then in alcohol (p. 33), and subsequently stained with Hansen's hematoxylin (p. 36). Mounted in damar, which clears vigorously, the finer details vanish.

No. 18.—*Elastic Cartilage.*—Take a piece of the arytenoid cartilage of man (better still of the ox)—the elastic cartilage of the anterior angle is recognized by its yellowish color. Cut a section that includes the boundary line between the elastic and hyaline cartilage, and examine it in water. Preserve like No. 17. The development of the elastic fibers may often be studied in the cartilages of adults, especially in the epiglottis and in the vocal process of the arytenoid cartilage (Fig. 32, 1).

No. 19.—*White Fibro-cartilage.*—Cut the intervertebral discs of adult man in pieces from 1 to 2 cm. square; fix in 100 c.c. of picrosulphuric acid (p. 21) for twenty-four hours and harden in 50 c.c. of gradually strengthened alcohols (p. 33). Stain sections in Hansen's hematoxylin (p. 36) and mount in damar (p. 45). Sections through the edges yield hyaline cartilage; through the central portions of the disc they exhibit large groups of cartilage-cells.

## III. THE MUSCULAR TISSUES.

The structural elements of the muscular tissues, the *muscle-fibers*, occur in two forms, the *smooth* and the *striated*. Both are cells, the body of which is extraordinarily elongated.

1. *Smooth, Non-striated, or Involuntary Muscle.*—The tissue of smooth muscle consists of contractile fiber-cells, spindle-shaped, cylindrical, or slightly-flattened elements with tapering extremities (Fig. 38).

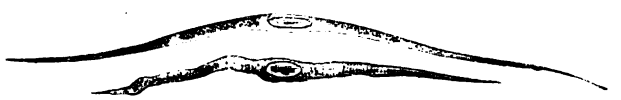

FIG. 38.—TWO SMOOTH MUSCLE-FIBERS FROM THE SMALL INTESTINE OF A FROG. × 240. Isolated in 35 per cent. potash-lye. The nuclei have lost their characteristic form through the action of the lye. Techn. No. 26.

They vary in length from 45 to 225 $\mu$, in width from 4 to 7 $\mu$; in the gravid uterus fibers measuring 0.5 mm. have been found. They are composed of a homogeneous protoplasm * and an elongated, elliptical, or rod-

* The protoplasm of certain fibers, those, for example, of the ductus deferens, exhibits longitudinal striation, which has led some authors to regard the smooth muscle-fiber as composed of minute contractile fibrillæ. In fishes and amphibians muscle-fibers containing pigment have been found in the iris.

shaped nucleus; the latter is characteristic of the smooth muscle-fiber. [Some authors add that the smooth muscle-fiber is invested by an exceedingly delicate, structureless, hyaline sheath, corresponding to the sarcolemma of the striated fiber.] The smooth muscle-fibers sometimes lie scattered in the connective tissue, sometimes are united in complexes.

FIG. 39.—INTERCELLULAR-BRIDGES OF SMOOTH MUSCLE-FIBERS. From a longitudinal section of the circular layer of the small intestine of a guinea-pig. × 420. Techn. No. 26 *b*.

In the latter case they are very firmly secured to one another by delicate thorn-shaped intercellular bridges (Fig. 39 and Fig. 40, A). Septa of connective tissue occur only at wider intervals (Fig. 40, B).

The fasciculi are united to form strata or membranes in which their disposition is parallel, as in the muscular coat of the intestine, or they

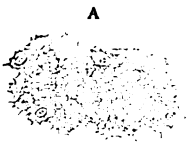


FIG. 40 A.—INTERCELLULAR BRIDGES. From a cross-section of the longitudinal muscular layer of the large intestine of a rabbit. × 600. Techn. No. 26 *b*.

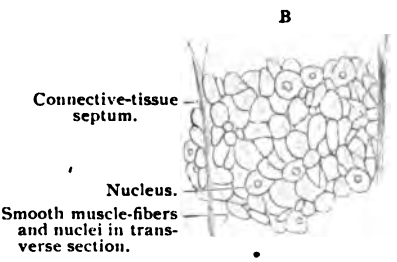


FIG. 40 B.—SECTION OF THE CIRCULAR LAYER OF THE MUSCULAR COAT OF THE HUMAN INTESTINE. × 560. The intercellular bridges cannot be seen. Techn. No. 103.

cross and interlace, forming complicated networks, as in the urinary bladder and the uterus. The larger blood-vessels run in the connective-tissue septa, the capillaries penetrate the fasciculi, within which they form networks with elongated meshes. The lymph-vessels follow the course of the blood-vessels and are present in considerable numbers.

For the nerves of smooth muscle, see the Peripheral Nerve-endings.

Smooth muscle-tissue occurs in the alimentary canal, in the trachea and bronchial tubes, in the gall-bladder, in the capsule and pelvis of the kidneys, in the ureters and the urinary bladder, in the reproductive organs, in the vascular channels and lymph-vessels, in the eye, and in the skin. The contraction of smooth muscle-fiber is slow and not under the control of the will.

2. *Striated or Voluntary Muscle.*—It is only by the study of their development that the striated muscle-fibers are recognized as the morphologic equivalents of cells. By a colossal growth in length, by proliferation of their nuclei, and by peculiar differentiation of their protoplasm, the embryonal elements have become highly specialized structures. The fibers are cylindrical in form and in the interior of the larger muscles have rounded or pointed ends; at the extremities of the muscle they possess a pointed inner end and a broad outer end, in contact with the tendon; the outer end is blunt or notched, often step-like and tapering. Anastomoses, divisions, and fissures occur; branched fibers are found in the muscles of the eye, the tongue, and the skin (Fig. 42, *4*). They vary in length from 5.3 to 12.3 cm., in width from 10 to 100 $\mu$. It is probable that there are fibers having greater length, but their isolation entire is very difficult to accomplish. In the embryo the fibers differ little in width, but after birth their development in this dimension varies and is dependent on the functional activity of the muscle; in the adult, robust muscles possess thick fibers, delicate muscles have thin fibers. Apart from this, their diameter depends on the nutritional condition of the individual. Furthermore, large animals possess thicker fibers than smaller ones. Hence the difference in caliber may be of a threefold nature.

Under the microscope each fiber exhibits alternate broad *dim* and narrower *clear* transverse striæ. The substance of the dim stripes is doubly refracting or *anisotropic*, that of the light stripes singly refracting or *isotropic*. High amplification shows that each transverse disc is transversely divided; invariably in the clear zone a dim line may be seen, the *intermediate disc*, and above and below this a dark band, the *accessory disc*, or *secondary disc*. In the anisotropic (dim) band a clear stripe, the *median disc*, has been observed. Owing to their extreme variation and their instability, these discs are of subordinate significance. Besides the cross-marking, a more or less distinct longitudinal striation may be observed. Treatment with chromic-acid solutions renders this striation more evident and even causes the muscle-fiber to fall apart into delicate longitudinal fibrils, each of which exhibits the cross-striæ.

These fibrils are the contractile structural elements of the muscle-fiber* and are called *ultimate fibrillæ*. They are grouped into bundles, the *sarcostyles* or *muscle-columns*, in which they are arranged parallel to one another and held together by the sarcoplasm, which also surrounds and unites the neighboring bundles. The disposition of the sarcoplasm is best seen in cross-section; high amplification is required. It presents the appearance of a clear network, within the meshes of which are the muscle-columns in section,—small, dark, polygonal areas known as *Cohnheim's fields*. The sarcoplasm contains the *interstitial granules*—consisting partly of fat and probably also partly of lecithin—and the *nuclei*. The latter are oval bodies placed parallel to the long axis of the

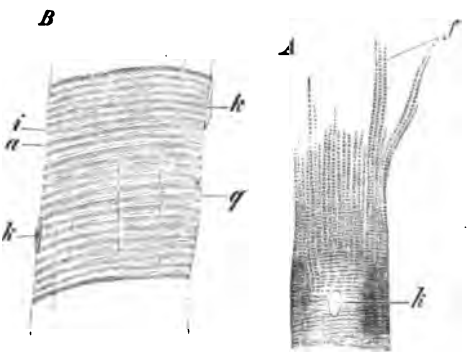


FIG. 41.—*B*. PORTION OF MUSCLE-FIBER OF MAN; *a*, anisotropic, *i*, isotropic band; *q*, intermediate disc; *k*, nucleus. × 560. Techn. No. 20 *b*. *A*. MUSCLE-FIBER OF FROG; *f*, fibrillæ; *k*, nucleus. × 240. Techn. No. 23.

muscle-fiber; in mammals, bony fishes, and some birds they are chiefly situated immediately beneath the sarcolemma, upon the surface of the muscle-substance; in other vertebrates they are embedded within the sarcoplasm.

Each muscle-fiber is closely invested by a structureless sheath, the *sarcolemma*, which represents the cell-membrane. Therefore the fiber of striated muscle consists of fibrillæ, sarcoplasm, muscle-nuclei, and sarcolemma.

The striated fibers are found in the muscles of the trunk and the extremities, of the eye and the ear, also in the tongue, the pharynx, the upper half of the esophagus, the larynx, the diaphragm, the genital organs, and the rectum.

* The muscle-fibers of some animals, after treatment with certain reagents, cleave transversely into discs. Fibrillæ and discs may be further separated into smaller prismatic, anisotropic particles called *sarcous elements*. Certain authors have interpreted the discs, others the sarcous elements, as the true structural units.

In some animals, the rabbit, for example, two varieties of striated muscles are distinguished, the *red* (semitendinosus, soleus) and the *white* or pale (adductor magnus); and correspondingly, two varieties of muscle-fibers: 1, dim fibers, rich in sarcoplasm, less regularly cross-striped, exhibiting more distinct longitudinal striation, possessing in general a smaller diameter (for example, those forming the soleus of the rabbit); 2, pale fibers, poor in protoplasm, more distinctly cross-striated, having in general a greater diameter. The latter represent the more highly differentiated muscle-fibers. While in certain animals the two varieties of fibers occur separately, each in particular muscles, in others —also in man—they are found intermingled in the same muscle. As a

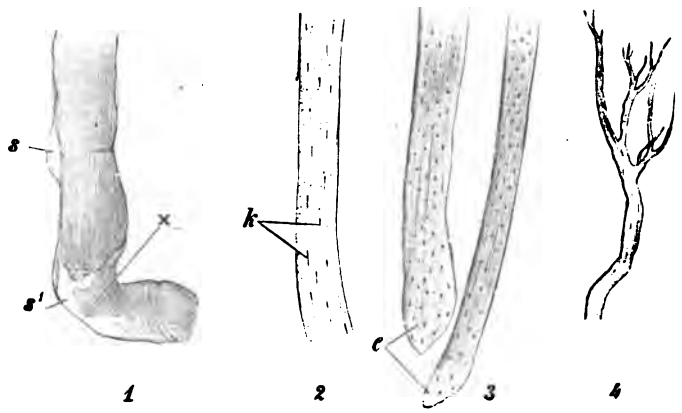


FIG. 42.—PORTIONS OF ISOLATED STRIATED MUSCLE-FIBERS OF FROG. × 50. 1. After treatment with water; $s^1$, sarcolemma; at $x$ the muscle-substance is torn, the cross-striation not apparent, the longitudinal striation distinct. Techn. No. 21. 2. After treatment with acetic acid: *k*, nuclei; the fine stippling represents the interstitial granules. Techn. No. 22. 3. After the action of concentrated potash solution; *c*, rounded ends; the numerous nuclei are swollen and vesicular in appearance. With this amplification the cross-striation in 2 and 3 is not visible. Techn. No. 24. 4. Branched muscle-fiber from the tongue of frog.

rule, the more functionally active muscles, the cardiac, ocular, masticatory, and respiratory, contain the greater number of red fibers. The pale fibers contract more rapidly, but are sooner fatigued.

The contraction of the striated fibers, compared with that of smooth muscle-fibers, is rapid and is under the control of the will. The striated fibers are united into bundles by fibrillar connective tissue, which serves also to convey the numerous ramifications of the blood-vessels and nerves supplying the muscular tissue. The lymphatic vessels are few in number.

3. *Cardiac Muscle.*—The muscle-fibers of the heart occupy a peculiar position. Although transversely striated, in the history of their development, as well as histologically, they must be regarded as modifi-

cations of the smooth muscle-fibers. In the lower vertebrates, in frogs, for example, they are spindle-shaped fibers possessing elongated nuclei, that often are more distinctly striated transversely than longitudinally (Fig. 43, *A*).

The cardiac muscle-fibers of mammals are short cylinders, the ends of which often are step-like. The protoplasm is partially differentiated into cross-striated *fibrillæ*, which not infrequently are grouped into *muscle-columns* radially arranged to the axis of the fiber (Fig. 43, *D*). The remnant of undifferentiated protoplasm, the *sarcoplasm*, proportionately considerable in comparison with that of striated voluntary fibers, is found chiefly in the axial part of the fiber, from which processes radiate between the muscle-columns. Owing to the generous amount and to the dispo-

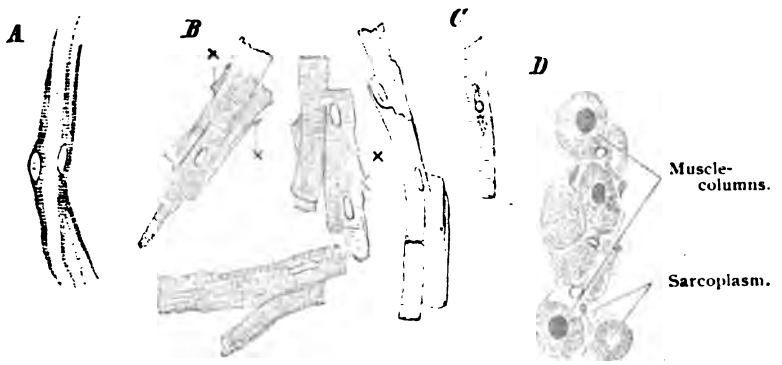


FIG. 43.—*A* and *B*, MUSCLE-FIBERS OF HEART, isolated in potash-lye. *A*, Of frog. *B*, Of rabbit; *x*, lateral branches. × 240. Techn. like No. 24. *C*, from a longitudinal section, *D*, from a cross-section of a papillary muscle of man. *C* magnified 240, *D* 560, diameters. Techn. No. 35.

sition of the sarcoplasm, longitudinal striation is often marked. The oval nucleus is embedded in the axial part of the sarcoplasm, which frequently contains pigment-granules or oil-droplets. A cell-membrane or sarcolemma is wanting. The cardiac muscle of the higher animals is characterized by the anastomosis of the cells by means of short, oblique or transverse, lateral processes. [The cells are joined end to end, transverse lines of cement-substance indicating the line of union between the individual elements.]

## TECHNIC.

No. 20.—*Striated Muscle-fibers; a (of the frog).*—With the scissors placed flat and parallel to the course of the fibers, cut a piece about 1 cm. long from the adductor muscle of a recently-killed frog. Take a fragment from the inner surface of this piece and tease it in a small drop of salt solution, add a second larger drop of the same liquid and, *without*

*pressing*, cover the preparation with a cover-glass. With low magnification (50 diameters) the cylindrical form, the difference in thickness, occasionally also the cross-striation of the isolated fibers may be seen (Fig. 42). With higher magnification (240 diameters) the cross-striation is distinctly seen, and occasionally pale nuclei and refracting granules. The presence of numerous granules within the muscle-fibers is probably an indication of active metabolic processes. Where the muscle-fibers are cut across, the muscle-substance not infrequently protrudes from the sarcolemma.

*b* (*Of man*).—I have found beautiful striated fibers in muscles taken from the human cadaver injected with carbolic acid.

To preserve, stain under the cover-glass with picrocarmine (p. 48) for about five minutes, then displace the staining fluid with diluted glycerol.

No. 21.—*The Sarcolemma.*—Treat preparation No. 20 *a* with a couple of drops of ordinary water. In from two to five minutes it will be seen, with the low power (50 diameters), that the sarcolemma is raised from the muscle-substance in the form of transparent blebs; at some places, where the torn muscle-substance has retracted, the sheath appears as a delicate line spanning the interval (Fig. 42, 1, *s s'*).

No. 22.—*Muscle Nuclei.*—Prepare muscle-fibers after No. 20 *a*; treat them with a drop of acetic acid (p. 48). The shrunken but sharply-outlined nuclei, with the lower power, have the appearance of spindle-shaped streaks (Fig. 42, 3).

No. 23.—*Fibrillæ.*—Place the fresh muscle of a frog in 20 c.c. of 0.1 per cent. chromic-acid solution (p. 30). In about twenty-four hours the tissue may be teased in a drop of water and fibers will be found, the ends of which have separated into their ultimate fibrillæ (Fig. 41, *A*). If it is desired to make a permanent preparation, place the muscle in water for one hour, then in 20 c.c. of 33 per cent. alcohol, ten or twenty hours; tease at once or preserve in 70 per cent. alcohol until wanted and then isolate (p. 28). If the chromic acid be removed by allowing the tissue to remain in alcohol (frequently renewed) for several weeks, the teased preparation may then be stained with picrocarmine in the moist chamber and this replaced by glycerol (p. 49). Beautiful fibrillæ can also be obtained by teasing the muscles of larval salamanders that have been fixed according to Techn. No. 1 and stained in bulk in borax-carmine (p. 37).

No. 24.—*The Ends of the Muscle-fibers.*—Place the fresh gastrocnemius muscle of the frog in 20 c.c. of concentrated potash-lye, and cover the watch-glass. In from thirty to sixty minutes (in a cold room, somewhat later) the muscle, if lightly moved with a glass-rod, falls into its fibers. Should this fail, the solution is not strong enough (see p. 29). Transfer a number of the fibers in a drop of the same solution to a slide and carefully apply a cover-glass. With the low power the ends of the

muscle-fibers and numerous nuclei may be seen (Fig. 42, 3). The fibers should not be examined in water or glycerol since the lye thus diluted soon destroys them.

No. 25.— *Branched Muscle-fibers.*— Remove the tongue from a recently-killed frog (it is attached in front to the lower jaw, is free behind) and place it in 20 c.c. of pure nitric acid, to which about 5 gm. of potassium chlorate have been added (some undissolved chlorate must remain in the bottom of the vessel). In a few hours, with glass-rods carefully transfer the tongue to 30 c.c. of distilled water, which must be frequently changed. In this the tissue can remain a week, though it may be used at the end of twenty-four hours. For this purpose put it in a test-tube half filled with water and shake it several minutes; the tongue will fall to pieces. Turn the contents of the test-tube into a capsule and in an hour or later place a little of the sediment that has been deposited in the meanwhile in a drop of water on a slide. The tissue may be further isolated with the teasing needles, but in most cases this is superfluous. Examine with the low power. Stain under the cover-glass with picrocarmine (p. 48). Mount in dilute glycerol (p. 22). (Fig. 42, 4.)

No. 26.—(*a*) *Smooth Muscle-fibers.*—These are best isolated by placing a piece of the stomach or intestine of a frog just killed in 20 c.c. of potash solution and treating like No. 25 (Fig. 38).

(*b*) *Intercellular Bridges.*—Take from a guinea-pig *just* killed a piece of the small intestine from 1 to 2 cm. long, fix it in 100 c.c. of Zenker's fluid (p. 31), harden it in gradually-strengthened alcohol (p. 33), and stain the sections with safranin (p. 25). The bridges can only be distinctly seen in very thin sections (5 $\mu$ thick) prepared with the microtome (see Paraffin Embedding, Microtome Technic).

## IV. THE NERVOUS TISSUES.

The elements of the nervous tissues, in an early embryonic stage, are without exception cells having a spherical form, the so-called *neuroblasts.* In the course of development they become elongated and pyriform; the narrow part grows out as a long, delicate process, often extending the length of a meter, and terminates in a free, branched end; it is named *nerve-process.* From the body of the cell, now termed a *nerve-* or *ganglion-cell*, other processes may arise, which, however, are short and divide dichotomously; they are called *dendrites*, or protoplasmic processes. Delicate lateral branches, the *collateral fibers*, may grow from the nerve-process. The nerve-cell and nerve-process together form an individual element, the *neuron* (neurodendron). The dendrites and collateral fibers are to be regarded as secondary processes of the neuron.

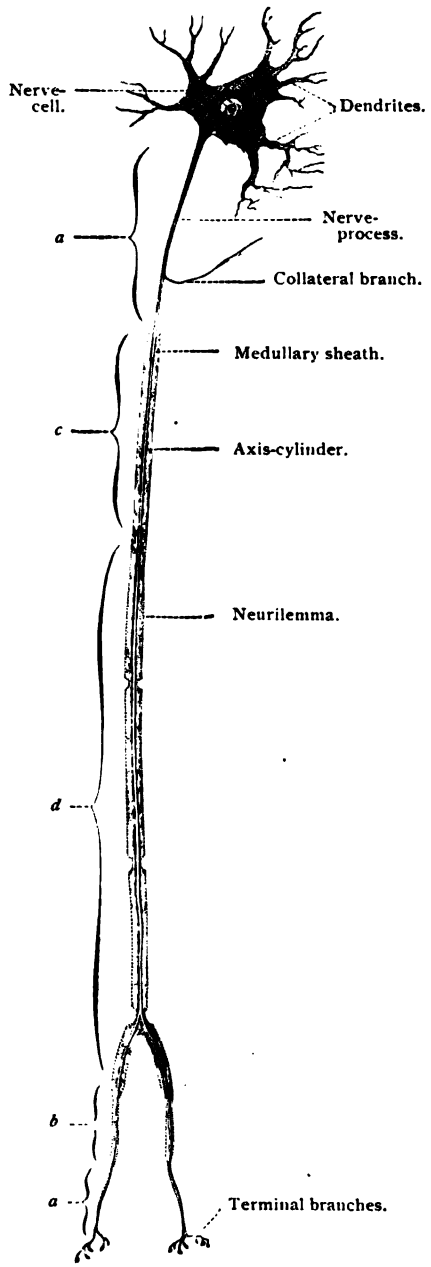


FIG 44.—DIAGRAM OF A NEURON.

The nerve-process may remain naked throughout its course, or it may receive different sheaths; these are the *neurilemma*, or sheath of Schwann, and the *medullary sheath.** Both invest the nerve-process only in a portion of its course. There are stretches in which the axis-cylinder is entirely without investment, is naked (Fig. 44, *a*); stretches in which it is enveloped only by the neurilemma (Fig. 44, *b*) or only by the medullary sheath (Fig. 44, *c*), and, finally, stretches in which both sheaths are present (Fig. 44, *d*); in this case the medullary sheath is always the innermost envelope, lies directly upon the cylindrical nerve-process, and is itself ensheathed by the neurilemma. The nerve-process always occupies the longitudinal axis; hence the name, *axis-cylinder*. Owing to the often great length of the nerve-process, it is not possible to investigate the neuron as a whole. As a rule, it is seen only in fragments, either the nerve-cell or the nerve-process, and this explains the former division of the elements of the nervous tissues into *nerve-cells* and *nerve-fibers*, the latter being the nerve-processes with their sheaths. There are no independent nerve-fibers, each so-called fiber is a process of a nerve-cell; if the connection between the fiber and the

* The neurilemma is a product of connective tissue; the origin of the medullary sheath requires further investigation; it is probable that the nerve-process and the nutritive fluid surrounding it play a part in its formation.

cell is broken, the fiber dies cellulifugalward from the point of solution of continuity. For practical reasons the old classification is retained.

## NERVE-CELLS.

Nerve-cells (ganglion-cells) are found in the ganglia, in the organs of special sense, along the course of cerebro-spinal, as well as sympathetic nerves, but principally in the central nervous system. They differ greatly in size (4 to 135 $\mu$ and more) and in form. There are spherical and spindle-shaped nerve-cells, and irregularly-stellate forms are very common; the latter are those in which the protoplasm sends off several processes and so gives rise to the stellate outlines. Nerve-cells having two processes are termed *bipolar*, those having several processes, *multipolar* ganglion-cells (Fig. 45 and Fig. 46). There are also *unipolar* nerve-cells; these occur in the sympathetic nerve of amphibians and universally in the olfactory mucous membrane. They possess, in fact, but a single process. The nerve-cells of the spinal ganglia, on the other hand, are only apparently unipolar; bipolar in the embryo, in the course of development they become unipolar by the gradual approach of the processes, which eventually come off from the cell by a common stalk, from which they then diverge at right or obtuse angles. These are the cells described as having T-shaped or Y-shaped processes. Apolar cells, that is, nerve-cells without processes, are either immature forms or artificial products, the processes in the latter case having been torn off in the manipulation required for isolation.

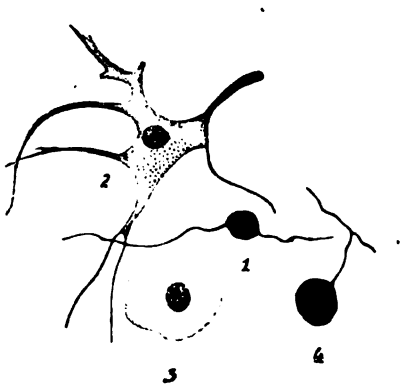


FIG. 45.—VARIOUS FORMS OF NERVE-CELLS. × 240. 1, Bipolar cell from the ganglion acusticum of an embryo rat. Techn. No. 187. 2, Multipolar cell from the spinal cord of man. Techn. No. 28. 3, Cell from the Gasserian ganglion of man, axis-cylinder process torn off. Techn. No. 27. 4, Cell with T-branches from a spinal ganglion of a young rat. Techn. No. 70.

Each nerve-cell consists of a granular or faintly-striated protoplasm,* that not infrequently contains pigment-granules, and of a vesicular

* The minute structure of the protoplasm differs in the different kinds of nerve-cells; for example, the motor-cells of the anterior horn of the spinal cord, besides pigment, possess deeply staining masses, the so-called *chromophilic granules*, that extend into the beginning of the dendrites, but not into the nerve-process. The protoplasm of the cells of the spinal ganglia, on the other hand, exhibits delicate filaments, that are in connection with granules.

nucleus poor in chromatin, that encloses a conspicuous nucleolus. This nucleus is characteristic for nerve-cells. A cell-membrane is wanting.

The processes of nerve-cells are of two kinds: 1, the *nerve-process* (axis-cylinder, axon) and, 2, the branched *protoplasmic* processes (dendrites). (Fig. 46 and Fig. 47.) They are most readily distinguished in the multipolar cells. The nerve-process, usually the only process of the kind,* is the first outgrowth from the embryonal spherical cell and is characterized by its hyaline appearance and smooth outlines; its course is cellulifugal—it leads from the cell. The protoplasmic processes, usu-

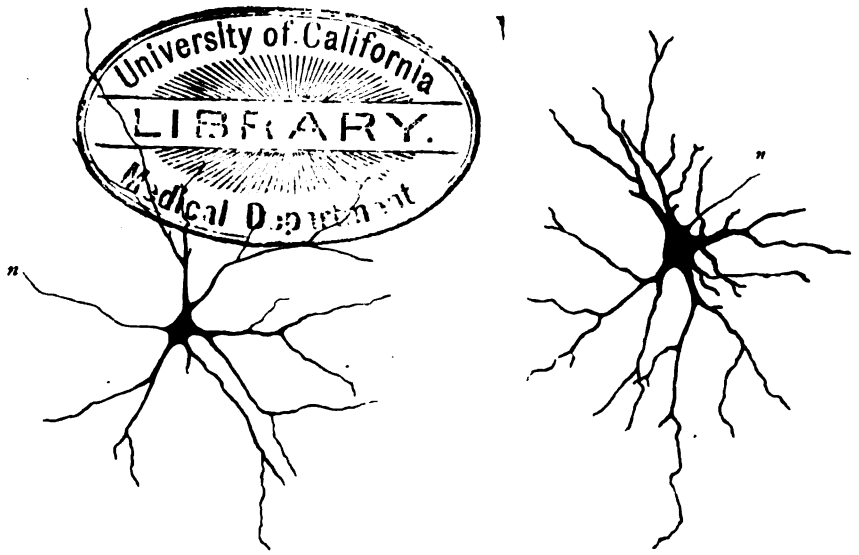


FIG. 46.—TWO FORMS OF MULTIPOLAR NERVE-CELLS FROM THE VENTRAL HORN OF THE SPINAL CORD OF A NEWBORN RABBIT, SHOWING THE RICHLY-BRANCHED PROTOPLASMIC PROCESSES. *n*. Nerve-process. 60. Techn. No. 70. (Schaper.)

ally several in number, are a later outgrowth of the embryonal cell, and are thicker, granular or finely striated, and often varicose; their course is cellulipetal—toward the cell. They undergo repeated division and finally terminate in an intricate arborization of extremely fine fibrils; in this way the cell acquires an enormous superficial enlargement, which on the one hand exalts the sustentative power, on the other, the susceptibility

* It is said there are cells with *several* nerve-processes, Cajal's cells in the cerebral cortex. In bipolar ganglion-cells, the two processes of which become the axis-cylinders of medullated nerve-fibers (cells of the spinal ganglia of lower vertebrates and of embryos) the central process running toward the central nervous system corresponds to the nerve-process, the peripheral process to a dendrite.

of the cell-body to nerve-stimuli—transmitted by the terminal ramifications of nerve-processes lying between the fibrils.

According to the behavior of the nerve-process, two kinds of nerve-cells are distinguished, *cells of the first type*, having a long nerve-process which becomes the axis-cylinder of a medullated nerve-fiber, and *cells of the second type*, having a short nerve-process which divides and subdivides and terminates in a nervous ramification in the vicinity of the cell (Fig. 48). The nerve-process of cells of the first type, after

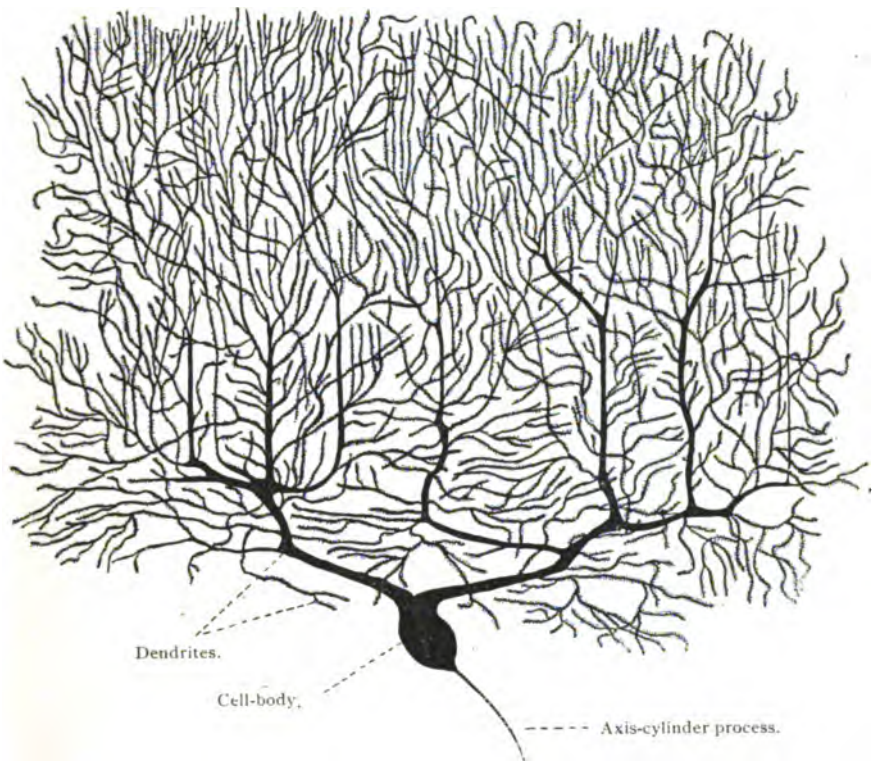


FIG. 47.—NERVE-CELL (CELL OF PURKINJE) FROM A SECTION THROUGH THE HUMAN CEREBELLAR CORTEX. × 180. Techn. No. 74.

giving off a number of fine, branched twigs, the collateral fibers (paraxons) and running an extended course, often embracing many centimeters, as the axis-cylinder of a nerve-fiber, undergoes rapid division and terminates in a plexus of delicate fibrils. It is supposed that all the processes terminate in free endings, without forming anastomoses; accordingly there is no connection between the processes of adjacent cells except by

contact. Properly, therefore, there can be no nervous network, but only a dense feltwork of interlacing fibrils * (*neuropilem*).

## NERVE-FIBERS.

Dependent upon the presence or absence of the medullary sheath, nerve-fibers are divided into *medullated*, or white, and *nonmedullated*, or gray. Each division is susceptible of a subdivision dependent on the presence or absence of the neurilemma.

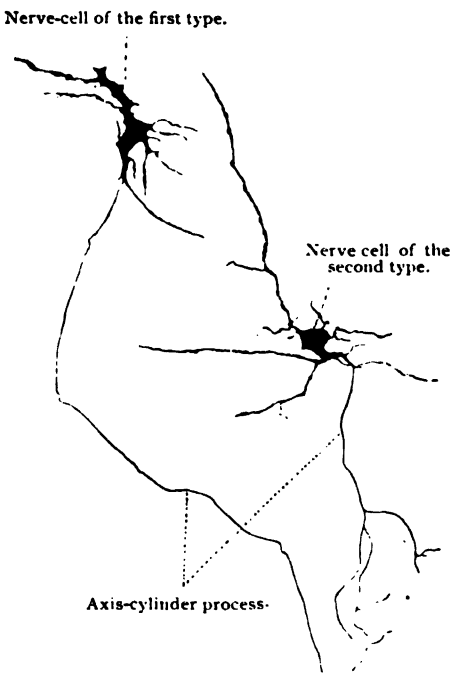


FIG. 48.—TWO NERVE-CELLS FROM THE SPINAL CORD OF AN EMBRYO CHICK SEVEN DAYS OLD. The axis-cylinder or nerve-process of the left cell is not seen in its entire length. × 200. Techn. No. 70.

**Nonmedullated Fibers.** *Without a Neurilemma.*—These fibers consist of the naked axis-cylinder alone and are found in the olfactory nerves, where they are held together and grouped into bundles by connective tissue. Similar are many fibers of the sympathetic nerve, the

* There may be some exceptions; in recent investigations of the retina and of the electric organ of the torpedo nervous networks formed by the processes of several nerve-cells have been described. In general, the phrase "nervous network" or "nervous plexus" is to be interpreted as signifying the disposition of single nerve-fibers that branch off from nerve-fiber bundles to join other bundles. The transition of one nerve-fiber into another never occurs.

so-called *Remak's fibers ;* * they are transparent, cylindrical or band-like in form, from 3 to 7 $\mu$ wide, about 2 $\mu$ thick, and exhibit faint longitudinal striation ; they are similarly grouped into bundles, which possess an imperfect sheath, formed by closely applied, flattened connective-tissue cells having oblong nuclei, that correspond to the endoneurium (see the chapter on the nervous system).

While the fibers so far described exhibit the same structure throughout their length, there are, on the other hand, nerve-fibers of which only certain divisions are naked axis-cylinders ; such divisions occur as peripheral endings of the nerves of special sense, of sensory as well as motor nerves ; also the first division of the nerve-process proceeding from the nerve-cell is a naked axis-cylinder (Fig. 44, *a*).

**Nonmedullated Nerve-fibers.** *With a Neurilemma.*—These consist of the axis-cylinder enveloped by a neurilemma and are of the same structure throughout their length ; they are found in many invertebrates and in cyclostoma. They occur as limited portions in the course of the cerebro-spinal nerve-fibers (Fig. 44, *b*).

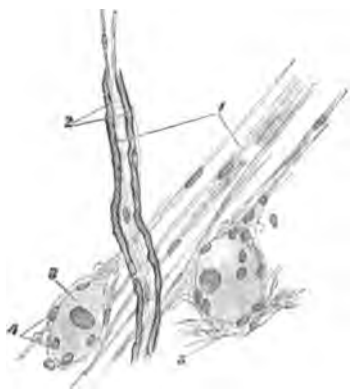

FIG. 49.—TEASED PREPARATION OF THE SYMPATHETIC NERVE OF RABBIT. 1. Nonmedullated, 2, thin medullated nerve-fibers; 3, ganglion-cell; the large nucleus has lost its characteristic appearance in consequence of the treatment with osmic acid; 4, nuclei of connective-tissue capsule; 5, fine connective-tissue fibers. × 240. Techn. No. 34.

**Medullated Nerve-fibers.** — Among these are no fibers that possess the medullary sheath in their entire length ; this always invests only one portion of the axis-cylinder. The medullated fibers may be *without a neurilemma*, and consist of the axis-cylinder and the medullary sheath ; such fibers occur only in the central nervous system. Medullated fibers *with a neurilemma* are found in the trunks and branches of the cerebro-spinal nerves, also in the sympathetic nerve, and vary in thickness from 1 to 20 $\mu$. The thickness of the nerve-fiber bears no relation to its motor or sensory nature, but appears to be determined by its length : the longer its course, the thicker is the fiber. Division of the medullated fibers occurs (1) throughout the central nervous system, principally where the collateral fibers diverge at right angles into the white substance ; and (2) in the peripheral nervous system shortly before their ultimate distribution (Fig. 44).

* By Remak's fibers some authors understand, not bundles of naked axis-cylinders, but individual axis-cylinder processes of sympathetic ganglion-cells.

The medullated nerve-fibers have a brief lease of life. They degenerate by a gradual breaking down of the medullary substance and axis-cylinder into a granular mass containing numerous nuclei; in this mass both parts are regenerated, the axis-cylinder probably by outgrowth of the axis-cylinder process of the nerve-cell. Regarding their finer structure and peculiar properties, the three constituent parts of nerve-fibers comport themselves in the following manner:

The **axis-cylinder,** the essential part of every nerve-fiber, occasionally exhibits a delicate longitudinal striation, the indication of its fibrillar structure. Each fibrilla represents a special conducting path

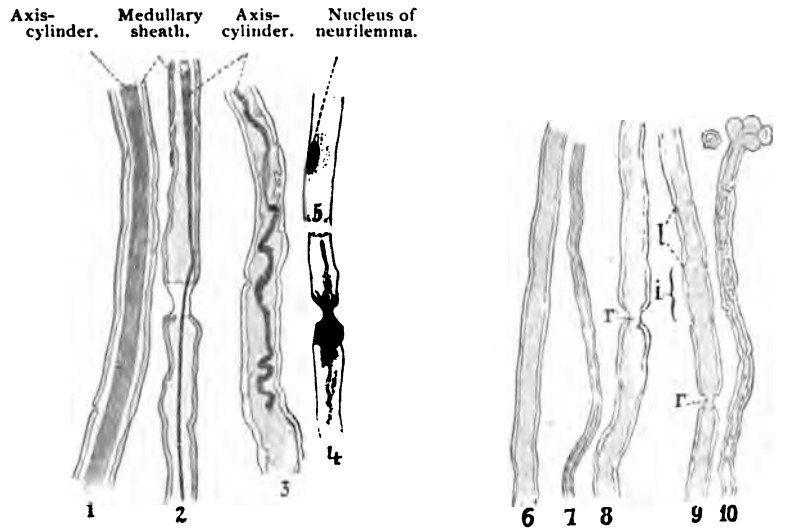


FIG. 50.—MEDULLATED NERVE-FIBERS FROM THE SCIATIC NERVE OF FROG. × 280. 1, Normal, 2, shrunken, 3, tortuous axis-cylinder; 4, node of Ranvier; 5, neurilemma with nucleus. Techn. No. 29. 6, 7, 8, and 9, Fresh medullated nerve-fibers; 10, post-mortem distortion of medullary substance; *r*, annular constriction; *l*, incisures of Lantermann; *i*, medullary segment. Techn. No. 29 *a*.

and is cemented to neighboring fibrillæ by a small amount of finely-granular interstitial substance, *neuroplasm.* [A delicate, elastic, special investment of the axis-cylinder, the axilemma, is described by Kühne. By some authors it is regarded as an artefact.]

The **medullary sheath** is composed of a semi-fluid, highly-refracting, fatty substance, the *myelin*, which imparts to fresh medullated fibers the appearance of glistening hyaline cylinders, homogeneous throughout, the structure of which can only be perceived by the help of reagents.

In favorable conditions it may be seen that the medullary sheath is not continuous, but is divided at slightly irregular intervals by oblique incisions or clefts into small conical or funnel-shaped pieces, the *Schmidt-*

*Lantermann segments* (medullary segments, cylindro-conical segments), which are united by cement-substance (Fig. 50, 9). Kölliker has interpreted these oblique markings as artefacts. After treatment with various reagents, the apparently homogeneous medullary substance of fresh nerve-fibers in dying undergoes partial transformation, and the fibers exhibit a characteristic double contour (thence the old designation, "double-bordered," or "dark-edged" fibers), and later appear mottled, owing to the distortion of the medullary substance, which collects into irregular spherical masses (Fig. 50, 10). [According to Kühne and Ewald the medullary substance consists of two parts: a reticulum composed of a resistant material resembling neurokeratin, which encloses within its meshes the other part, the myelin. Owing to the variability in the appearance of the network, other authorities regard it as an effect of the reagents employed to demonstrate it.]

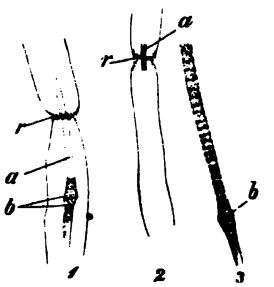


FIG. 51.—MEDULLATED NERVE-FIBERS OF FROG, TREATED WITH SILVER NITRATE SOLUTION. × 560. 1. At *r*, node of Ranvier; *a*, axis-cylinder, of which only a small extent is silvered; *b*, biconical swelling displaced downward owing to manipulation. 2. Axis-cylinder with the silvered portion *in situ*, at *a*. 3. Axis-cylinder with cross-markings. Techn. No. 33.

At regular intervals along the medullated nerve-fibers the medullary substance is interrupted, so that the axis-cylinder and neurilemma come into contact. At these points the fibers exhibit well-marked annular constrictions, termed *nodes of Ranvier* (Fig. 51); they are the localities where the nutritive fluid can approach the axis-cylinder. These constrictions occur in all peripheral medullated fibers, at intervals of from 0.08 mm. in thin, to 1 mm. in thick fibers, dividing them into *internodal segments* or *internodes*. In the vicinity of the nodes the axis-cylinder frequently shows a biconical enlargement, probably due to a local accumulation of neuroplasm. After treatment with silver nitrate, the nodes are rendered conspicuous by a dark annular disc called the constricting band, produced by the staining of the cement-substance collected at these points, and by distinct transverse striæ (Frommann's lines), that appear on the adjacent parts of the axis-cylinder.*

The *neurilemma*, or sheath of Schwann, is a delicate structureless membrane, against the inner surface of which lie oval nuclei surrounded by a very small amount of protoplasm (Fig. 50, 5).

The union of the elements of the nervous tissues in the peripheral

* These striæ are artefacts; for their significance, see p. 40, remark *.

nervous system is secured by means of connective tissue, which contains the ramifications of the blood-vessels. In the central nervous system they are supported and held together, not only by connective tissue, but by a peculiar form of tissue, the *neuroglia.*

## TECHNIC.

No. 27.—*Ganglion-cells, fresh.*—Tease a small piece of the Gasserian ganglion in a drop of salt solution and stain under the cover-glass with picrocarmine for two minutes (p. 48). The processes of the cells usually tear off.

The nerve-cells of the cerebral and the cerebellar cortex may be prepared in the same way; the processes likewise are easily lost.

No. 28.—*Multipolar Nerve-cells of the Spinal Cord.*—Remove with the scissors as much as possible of the white substance of the spinal cord of an ox and place the gray remnant in pieces 1 or 2 cm. in length in 30 c.c. of 33 per cent. alcohol (p. 20, *e*). After thirty-six or forty-eight hours transfer the pieces to 20 c,c. of undiluted neutral carmine solution (p. 24) for twenty-four hours. The now very soft pieces should be transferred with the section-lifter to 50 c.c. of distilled water, in order to wash out some of the stain, and after ten minutes spread with needles in a thin layer on a dry slide. With a little practice the nerve-cells can be distinguished by their bright-red nuclei; the cell-body and the processes are not yet visible. Let the preparation dry thoroughly and mount it in damar (Fig. 45, 2).

No. 29.—*Fresh Medullated Nerve-fibers.*—Expose the sciatic nerve of a frog just killed, and with delicate scissors cut it at the level of the popliteal space and about one cm. higher. Isolate in a drop of salt solution.

No. 29 *a.*—Better still, tease on a dry slide by the "half-drying" method. Hold the lower end of the nerve with one needle, with another needle separate the nerve-bundles along half the length of the nerve; a thin shining membrane will span the interval between the separated bundles. Add a drop of salt solution and apply a cover-glass. The membrane contains numerous isolated nerve-fibers. The manipulation must be done very rapidly (in about fifteen seconds), so that the nerve-fibers do not become dry (Fig. 50, 6, 7, 8, 9).

No. 30.—*Alterations of the Medullary Sheath.*—Treat No. 29 *a* with water (place a drop at the edge of the cover-glass and let it flow under). In a few minutes the formation of the myelin drops begins (Fig. 50, 10).

No. 31.—*The Axis-cylinder.*—Tease dry (like No. 29 *a*) and stain with methylene-blue (p. 39); the nodes of Ranvier stain first, and often so deeply that the axis-cylinder cannot be recognized there (Fig. 50, 4). The axis-cylinder frequently shrinks and becomes displaced within the

medullary sheath, or it contracts and becomes convoluted (Fig. 50, 2, 3). On the addition of glycerol the medullary substance can no longer be distinctly recognized as such, but the nuclei of the neurilemma are often rendered plainly visible (Fig. 50, 5).

No. 32.—*Exhibition of the Axis-cylinder with Chromic Acid.*—Expose the sciatic nerve of a rabbit recently killed, *being careful not to touch it;* place a match-stick parallel to the long axis of the nerve and secure it by means of ligatures at the upper and lower ends; cut the nerve on the farther side of each ligature and place it, with the wood, in 100 c.c. of a 0.1 per cent. chromic-acid solution (p. 31).

In about twenty-four hours cut the ligatures and tease a piece of the nerve, from 0.5 to 1 cm. long, separating it into bundles, not fibers. Put the bundles back into the chromic-acid solution; after twenty-four hours transfer them to 50 c.c. of distilled water, and after two or three hours to 30 c.c. of gradually-strengthened alcohols to harden (p. 33). It is advantageous to leave the bundles for a long time, one to eight weeks, in 90 per cent. alcohol, as they are then more readily stained. After the hardening is completed, the bundles are to be teased in a drop of picrocarmine, placed in the moist chamber, and, after the staining is completed (which according to the length of time the tissue was allowed to harden in the alcohol requires from one-half to three days), preserved in acidulated glycerol (p. 49). The nodes of Ranvier are not as distinct as in fresh and in osmic-acid preparations, but appear as delicate transverse lines (Fig. 52). The somewhat shrunken axis-cylinder and the nuclei are stained a fine red. The intensity of the color depends on the quality of the picrocarmine, which unfortunately varies greatly.

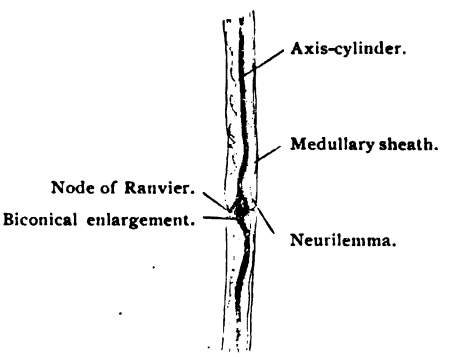


FIG. 52.—NERVE-FIBER OF RABBIT. × 560.

No. 33.—*Nodes of Ranvier and Axis-cylinders.*—Add 10 c.c. of a 1 per cent. solution of silver nitrate to 20 c.c. of distilled water. Kill a frog, open the abdomen by a crucial incision, turn out the viscera, and expose the nerves descending on either side of the vertebral column. Wash out the abdominal cavity with distilled water and pour over the nerves about one-third of the silver solution. After two minutes carefully cut out the delicate nerves, put them for a half-hour in the remainder of the silver solution, and *place them in the dark.* Then transfer them to 10 c.c. of distilled water, in which they may remain for from one to twenty-four hours. If the nerves are now examined in a drop of water, with the low power, the endothelial sheath of the nerve and numerous pigment-cells will be seen; frequently a blood-vessel lies along the nerve.

On examination with the high power, little will be seen of the nodes and axis-cylinders, but if the preparation be exposed for several hours to daylight (or a few minutes to sunlight) the reaction takes place and the parts mentioned become silvered. The biconical swelling on the axis-cylinder often becomes displaced in teasing, and is not always readily found by the beginner (Fig. 51).

No. 34.—*Nonmedullated Nerve-fibers.*—Tease a portion of the pneumogastric nerve of a rabbit on a dry slide (No. 29 *a*), and add a few drops of a 0.5 per cent. osmic-acid solution; in five or ten minutes the medullated nerve-fibers become blackened (which may be ascertained by examination with the low power). Remove the osmic-acid solution and add a few drops of distilled water, which should be renewed in five minutes. In five minutes more remove the water, add a few drops of picrocarmine, apply a cover-glass, and place in the moist chamber for from twenty-four to forty-eight hours; then displace the picrocarmine with acidulated glycerol (p. 49). The tissue may be teased again after the staining is completed, which is now more easily done because the elements are more distinctly seen. With high magnification the medullated nerve-fibers appear blue-black, the nonmedullated pale gray and finely striated longitudinally. The sympathetic nerve treated in the same way exhibits more numerous nonmedullated nerve-fibers. But this nerve is somewhat more difficult to find. Cut through the greater cornu of the hyoid bone, also the hypoglossal nerve, and push them aside; behind the pneumogastric nerve lies the sympathetic, which is recognized by its three or four mm. in size, ellipsoidal, yellowish, and transparent superior cervical ganglion. If the piece of the nerve lying close under the ganglion be teased, ganglion-cells, the majority of which contain two nuclei, will be obtained; it is difficult to isolate the cells so that their processes can be seen. In Fig. 49, accidentally, only the more unusual uninucleated ganglion-cell is seen.

# II. MICROSCOPIC ANATOMY OF THE ORGANS.

## 1. THE CIRCULATORY SYSTEM.

### THE BLOOD-VESSELS.

The blood-vessels are composed of fibrous connective tissue, elastic fibers, and smooth muscle-fibers, mingled in widely different proportions and arranged in strata or tunics. In general, a uniform disposition of the elements prevails in each tunic; longitudinal in the inner and outer, circular in the middle tunic. An exception to this occurs in the complicated structure of the heart and in the simple structure of the capillaries.

#### The Heart.

The walls of the heart consist of three membranes: 1, the endocardium; 2, the powerfully developed muscular layer, the myocardium; 3, the epicardium (visceral layer of the pericardium).

The *endocardium* is a connective-tissue membrane which contains smooth muscle-fibers and numerous elastic fibers. The latter are especially well developed in the auricles, where they form a close-meshed network or are blended in a fenestrated membrane (Fig. 24). The free surface, that directed toward the cavity of the heart, is clothed with a simple layer of irregularly-polygonal epithelial- (endothelial) cells.

FIG. 53. — CROSS-SECTION OF PAPILLARY MUSCLE OF HUMAN HEART. *m*, Muscle-fibers in section; *p*, perimysium with small deeply-stained nuclei; *v*, blood-vessel. × 240. Techn. No. 35.

The *muscular layer* or *myocardium* consists of muscle-fibers (for their structure, see p. 95) and a delicate perimysium surrounding each element. Numerous transverse and oblique processes (see Fig. 43) unite the muscle-fibers into complexes, the arrangement of which is very complicated. The muscle-tissue of the auricles is entirely separate from that of the ventricles. In the auricles an outer transverse layer common to both and an inner longitudinal layer independent in each can

be distinguished. In addition, numerous small bundles pursue independent courses in other directions. The muscle-tissue of the ventricles is much more irregularly distributed; the bundles extend in every direction, often describing a figure-of-eight in their course.

Within the compass of the auricles the perimysium contains elastic fibers, that are connected with those of the endocardium and of the epicardium. The muscular layer of the auricular appendages is poor in elastic tissue. Between the auricles and ventricles lie firm tendinous ligaments intermingled with elastic fibers, the *annuli fibrosi*, of which the right is stronger than the left. Similar but less developed ligaments lie at the arterial orifices of the ventricles. Numerous muscle-fibers take their origin in these ligaments.

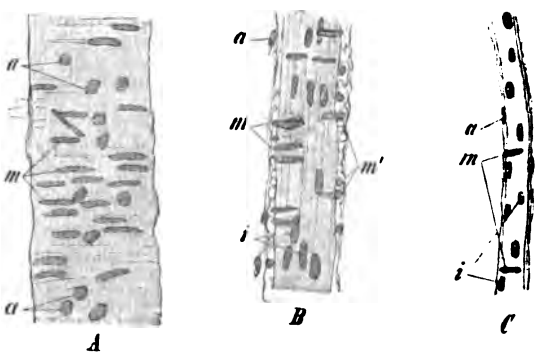


FIG. 54.—SMALL ARTERIES OF MAN. *i*, Nuclei of intima, the outlines of the cells are invisible; *m*, nuclei of circularly-disposed muscle-fibers of media; *a*, nuclei of externa. *A*, artery with the surface in focus. *B*, artery with the lumen in focus; at *m'* the nuclei of the muscle-fibers of the media are seen in optical section. *C*, small artery shortly before transition into capillaries; the media consists of a few isolated muscle-fibers. × 240. Techn. No. 37 *a*.

The *epicardium* is a connective-tissue membrane penetrated by elastic fibers and fat-cells, which on the outer surface is covered with a single stratum of squamous epithelium.

The *valves* of the heart are composed of fibrous connective tissue, continuous with that of the annuli fibrosi, and their surfaces are clothed by the endocardium. They contain muscle-fibers, but only in the attached margin, and elastic fibers, which are especially abundant in the nodules of the free edges of the semilunar valves.

The numerous *blood-vessels* of the muscular wall of the heart form typical capillary networks with elongated meshes (see the Organs of Muscular System). The epicardium and endocardium, the latter in its deeper strata, also possess blood-vessels.

The *lymph-vessels* of the heart are extremely numerous. They form a comprehensive system embracing all the lymph-spaces in the

clefts between the muscle-fibers and accompany the blood-vessels in their course.

The *nerve-supply* of the heart includes medullated nerve-fibers derived from the pneumogastric and nonmedullated sympathetic nerve-fibers from the cervical ganglia ; along their course numerous ganglion-cells occur.

The *pericardium* consists of compact connective tissue intermingled with elastic fibers, which on its inner surface, that directed toward the heart, is clothed in a simple layer of squamous epithelium.

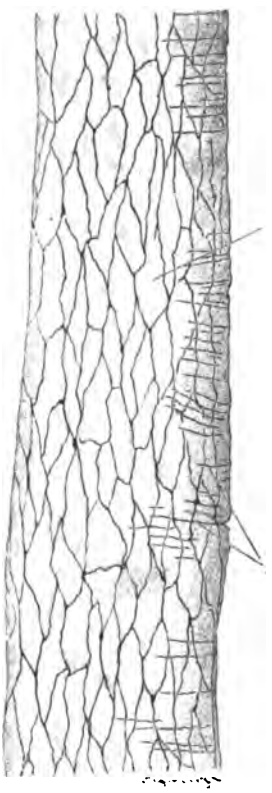


FIG. 55.—ENDOTHELIUM OF A MESENTERIC ARTERY OF RABBIT. Surface view. × 260. Techn. No. 38.

## THE ARTERIES.

The walls of the arteries comprise three coats: 1, tunica intima; 2, tunica media; 3, tunica externa (adventitia). The elements of the tunica media are transversely disposed, those of the other tunics chiefly longitudinally. The structure and thickness of these coats vary with the size of the artery. This renders their classification as small, medium, and large arteries desirable.

The *small arteries* include the terminal branches shortly before their transition into capillaries. The *intima* consists of elongated, spindle-shaped epithelial-cells and a structureless elastic membrane, the so-called *internal elastic membrane*, that in somewhat larger arteries assumes the character of a fenestrated membrane. The *media* is formed of a single layer of circularly-disposed smooth muscle-fibers. The *externa* is composed of longitudinally-disposed bundles of connective tissue and fine elastic fibers. It blends insensibly with the surrounding connective tissue.

The *arteries of medium size* comprise all the named arteries of the body with the exception of the aorta and the pulmonary artery. The *intima* of these vessels has increased in thickness owing to the interposition between the endothelium and internal elastic membrane of fibrous connective tissue, including flattened cells and networks of delicate elastic

fibers.* The *media*, in addition to several superimposed layers of circularly-arranged smooth muscle-fibers, comprises wide-meshed networks of elastic fibers. At the inner boundary of the media of some arteries longitudinally-disposed muscle-fibers occur; these are especially well developed in the subclavian artery. The proportion of the two tissues in the different arteries is extremely variable. In the celiac, femoral, and radial arteries the muscle-tissue preponderates; in the

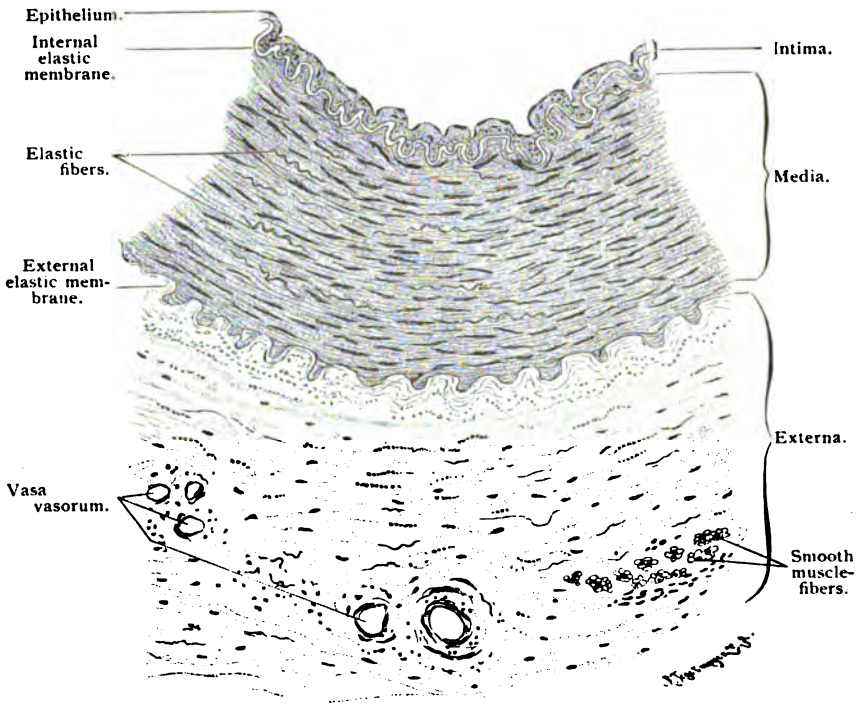


FIG. 56.—PORTION OF CROSS SECTION OF THE BRACHIAL ARTERY OF MAN. × 100. Techn. No. 35.

carotid, axillary, and common iliac, the elastic tissue is in excess. The externa has also become stouter. Thick elastic fibers occur in especial profusion at the boundary of the media and in many arteries form a continuous layer designated the *external elastic membrane*. New elements in the externa of arteries of medium size are smooth muscle-fibers, that appear in single, longitudinally-disposed bundles and never form a continuous layer.

In the *large arteries* (aorta and pulmonary artery) the epithelial-

* This subendothelial layer is absent in the uterine arteries of young individuals, in the celiac, the external iliac, the renal, and the mesenteric arteries.

cells of the *intima* are shorter and more polyhedral in outline than in medium-sized vessels. Immediately beneath is the subendothelial layer, that consists of fibrous connective tissue enclosing elastic networks and flattened cells, stellate or spherical in outline. The elastic networks are closer meshed the nearer to the intima they lie, and finally pass into a fenestrated membrane corresponding to the internal elastic membrane of small- and medium-sized arteries. The *media* of the large arteries is

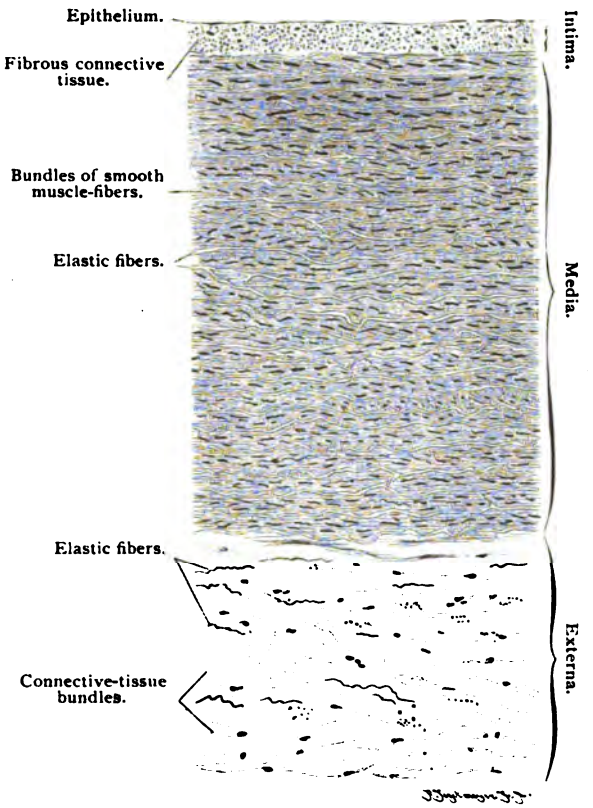


FIG. 57.—FROM A CROSS-SECTION OF THE THORACIC AORTA OF MAN. × 100. Techn. No 35

characterized by the preponderance of elastic tissue over the muscular elements. The thin elastic networks of the media of medium-sized arteries are here replaced by close networks of richly-developed, broad, elastic fibers or by fenestrated membranes, which alternate regularly with lamellæ of smooth muscle-fibers. The elastic elements, like the muscle-fibers, are circularly arranged. The muscular lamellæ are penetrated in an oblique direction by elastic fibers which connect all the elastic elements of the media.

The elastic membranes already occur in the larger medium-sized arteries; they are especially well-marked in the carotids, which closely approach in structure the large arteries. The *externa* of large arteries presents no essential peculiarity and differs but slightly from that of medium-sized arteries. It does not possess the external elastic membrane. In the lower animals smooth muscle-fibers are present.

The foregoing classification of the strata of the wall of the artery corresponds to present usage. There is a new proposition to regard as intima simply the endothelial tube alone, as externa all that lies outside of the external elastic membrane, the latter to be reckoned as belonging to the media. Between these two, then, lies the media, of which the

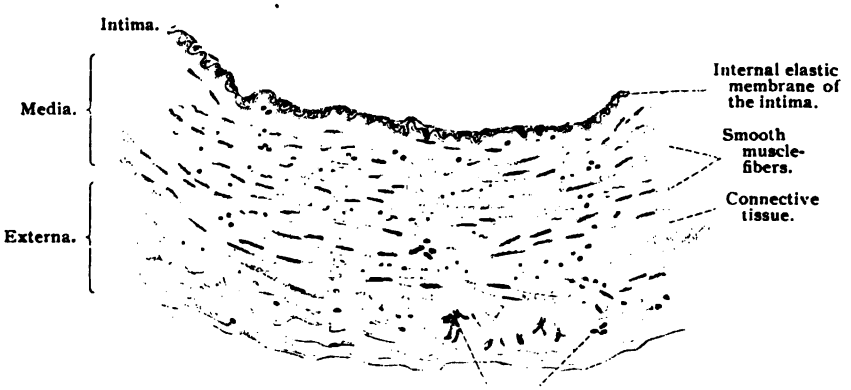


FIG. 58.—PORTION OF CROSS-SECTION OF A VEIN OF LIMB OF MAN. × 100. Techn. No. 35.

external and internal elastic membranes represent the border-lamellæ. The subendothelial layer of the large arteries is to be reckoned as belonging to the media.

## THE VEINS.

There is no definite proportion between the size of the veins and the thickness of their walls, and no basis for a division into groups as in the arteries. The veins are characterized by the preponderance of fibrous connective tissue and by the slighter development of the muscular elements. As in the arteries, three coats may be distinguished.*

The *intima* consists of a single layer of endothelial cells, that are fusiform only in the smallest veins, in others are polygonal in form. In

* Owing to the meager development of the media some histologists have suggested that only two coats are present, tunica intima and tunica externa, and that the layers usually regarded as tunica media belong to the latter.

veins of medium size, having a diameter of from two to nine mm., the subendothelial layer consists of connective tissue containing nucleated cells, that in large veins (femoral, popliteal, superior cava) develops in the form of distinct fibrous lamellæ. Surrounding this is the internal elastic membrane, which is structureless in small veins, in medium-sized and large veins is represented by elastic networks. Obliquely or longitudinally-disposed smooth muscle-fibers occur in the intima of the iliac, femoral, saphenous, and mesenteric veins.

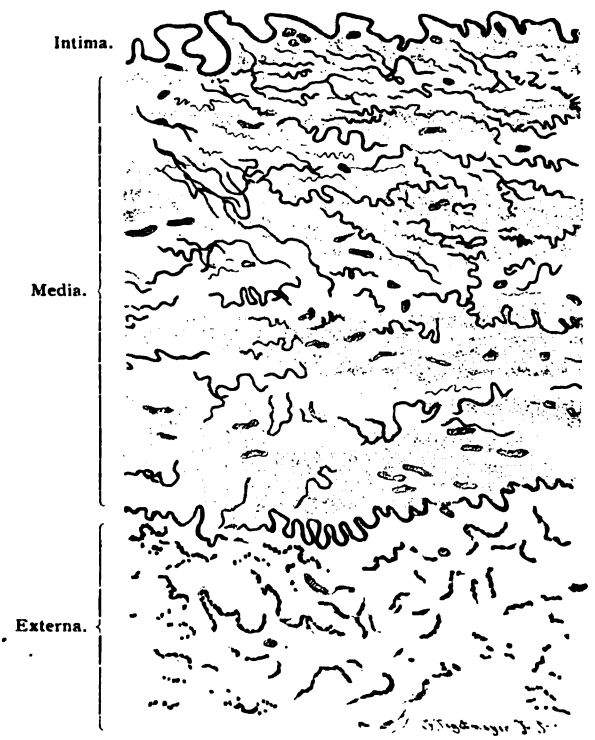


FIG. 59.—CROSS-SECTION OF A VEIN OF A HUMAN LIMB. × 420. The elastic elements are stained. Techn. No. 36.

The *media* exhibits great variation. It is composed of circular muscle-fibers, elastic networks, and fibrous connective tissue, and is best developed in the veins of the lower extremities (especially in the popliteal), less so in the veins of the upper extremities, still less in the large veins of the abdominal cavity; it is absent in many veins (those of the pia and dura, of the bones, of the retina, in the superior cava, and also in the veins proceeding from the capillaries).

The usually well-developed externa consists of intercrossing bundles of connective tissue, elastic fibers, and longitudinally-disposed smooth muscle-fibers, that are more highly developed in the veins than in the arteries. The adventitia of certain veins (the trunk of the portal and the renal) possesses an almost complete membrane of longitudinally arranged muscle-fibers (Fig. 60).

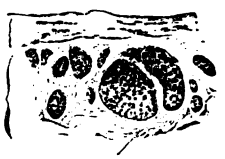


FIG. 60.—CROSS-SECTION OF THE RENAL VEIN OF MAN. × 50. Techn. No. 35.

The *valves* of the veins are folds of the intima covered on both surfaces by epithelial-cells, that on the surface directed toward the vascular stream are elongated in the direction of the current; on the opposite surface, toward the wall of the vein, they are transversely elongated.

## THE CAPILLARIES.

The capillaries establish the communication between the arteries and veins. There are a few exceptions, as, for example, in the corpora cavernosa of the genital organs. The transition of the arteries into the capillaries is effected by a gradual simplification of the structure of the vessel-wall (Fig. 54). The media becomes steadily thinner and finally is represented by a few isolated circularly-disposed muscle-fibers occurring at wide intervals, that ultimately disappear. The externa becomes correspondingly attenuated until it consists of a thin layer of connective tissue containing cells, that ultimately also vanishes, so that at last the only part of the vessel-wall that remains is the intima, the layers of which are likewise reduced until nothing is left but a stratum of plate-like, nucleated endothelial cells. Hence, the walls of the capillaries consist of a simple layer of endothelial cells, the form of which may be most aptly compared with a steel pen pointed at both ends. These cells are united at their edges by a small amount of cement-substance.

The capillaries divide without decrease in caliber and by anastomosis with neighboring capillaries form networks differing widely in the size of the meshes. The closest meshes occur in the capillary networks of secretory organs, for example, in the lung and liver; wide-meshed networks occur in the muscles, the serous membranes, the special-sense organs. The reverse obtains in regard to the caliber of the capillaries; the widest capillaries are found in the liver, the narrowest in the retina and in the muscles.

*Development of Capillaries.*—Only the developmental processes in

the post-embryonic epoch will be considered here. A minute, conical, protoplasmic mass appears on the wall of an existing capillary, resting by a broad base on the latter and terminating in a slender, tapering, free end.* In the further course of development this pointed free end unites with another off-shoot that has arisen in the same way from another point on the capillary wall. These formations are solid at first, but gradually become hollow by an extension of the lumen of the capillary, and subsequently the walls of the new vessels become differentiated to endothelial cells. The development of new capillaries is always consummated in connection with existing capillaries.

All medium and large blood-vessels possess small blood-vessels

FIG. 61.—SURFACE VIEW OF A PORTION OF THE GREATER OMENTUM OF A SEVEN-DAY-OLD RABBIT. *c*, Blood-capillaries, some containing blood-corpuscles; *s*, capillary sprout tapering to a free solid point; *i*, young capillary, the greater part of which is hollow, at *s'* still solid; *k*, nuclei of peritoneal endothelium. × 240. Techn. No. 40.

(vasa vasorum) that provide for the nutrition of their walls; they run almost exclusively in the adventitia (Fig. 56). The intima always is without blood-vessels.

All blood-vessels are furnished with nerves, which form a plexus of medullated fibers in the media of the arteries and veins. From these, nonmedullated fibers arise which are distributed to the muscle-fibers. The capillaries are accompanied by encircling networks of delicate nonmedullated nerve-fibers. Many blood-vessels are surrounded by lymph-channels; occasionally the lymph-spaces in the adventitia are so wide that they form an ensheathing sinus for the blood-vessel, the adventitial or perivascular lymph-space.

* Such blind capillary sprouts may be hollowed out at an early period; corpuscles that happen to flow into them disintegrate, because they are excluded from the circulation and the interchange of gases, and fall into fragments that have been erroneously interpreted as hematoblasts; they have no connection with the true hematoblasts.

The *glomus caroticum* ("glandula carotica") is no gland, but consists essentially of blood-vessels. The capillaries arising from the division of the single arterial vessel differ greatly in width and are surrounded by numerous cells resembling the plasma-cells of connective tissue, that are arranged in rounded groups forming the so-called secondary nodules. The many veins collect at the periphery of the organ, that besides contains fibrous connective tissue, isolated ganglion-cells, and conspicuous numbers of medullated and nonmedullated nerve-fibers. Similar in structure is the coccygeal gland (*glomus coccygeum*), the blood-vessels of which are characterized by hemispherical evaginations.

## The Blood.

The blood * is a slightly clammy, red-colored liquid which consists of a fluid substance, the *blood-plasma*, and *formed elements*, the blood-cells,

FIG. 62.—BLOOD-CORPUSCLES MAGNIFIED 560 TIMES. *A*. Of man: 1–6, discoidal colored blood-cells; 1, seen with close focus; 2, with distant focus; 3 and 4, viewed edgewise; 5, crenated in consequence of evaporation; 6, after treatment with water; 7, spherical colored blood-corpuscle; 8, colorless blood-corpuscle; 9, blood-platelets. *B* Of frog: 10–13, colored blood-cells; 10, fresh, nucleus indistinct; 11, a few minutes later, nucleus plainly visible; 12, seen from the side; 13, after treatment with water; 14, living, 15, dead colorless blood-corpuscles. Techn. No. 41, 43, 44.

the blood-platelets, and the elementary granules. The blood-cells are of two kinds: colored blood-cells and colorless blood-cells.

The *colored blood-cells* are soft, flexible, highly-elastic elements and possess smooth, slippery surfaces. In man and in other mammals they have the form of a flat, circular disc,† slightly concave on each surface, and therefore resemble biconcave lenses. Exceptions occur in the llama and the camel, in which the colored blood-cells are oval. The average diameter in man is 7.5 $\mu$, the thickness 1.5 $\mu$. The colored blood-corpuscles of domesticated mammals are all smaller; the largest are those of the dog (7.3 $\mu$). The colored blood-cells consist of a *stroma* (proto-

* The elements of the blood do not form a tissue, but represent a loose union of elementary parts, without definite arrangement of the same,—an aggregation of cells.

† In addition, there occur in human blood *spherical* colored blood-corpuscles, Fig. 62, A, 7; they are smaller (5 $\mu$) and few in number.

plasm), the spaces of which are filled with the blood-coloring matter, the *hemoglobin*. The hemoglobin imparts to the corpuscle its yellow or yellowish-green color. A nucleus and a proper cell-membrane are wanting. The colored blood-corpuscles of fishes, amphibians, reptiles, and birds are distinguished from those of mammals by their oval, biconvex form, their generally greater size (22 $\mu$ long by 15 $\mu$ broad in the frog), as well as by the presence of a round or oval nucleus; in other respects they exhibit the same properties as those of mammals.

The *white or colorless blood-cells* (leucocytes) occur not only in the blood but also in the lymphatic vessels, where they are termed "lymph-corpuscles." They are also found outside of the vessels, in bone-marrow, in masses in adenoid tissue, scattered in fibrous connective tissue, and between epithelial- and gland-cells, where they have wandered by their power of ameboid movement; therefore they are also called "wandering cells."

In all cases the colorless blood-cells consist of a clammy protoplasm and a nucleus, and are without a cell-membrane. A definite form cannot be described, because during life they are engaged in ameboid activity. In a state of rest they are spherical (Fig. 63).

FIG. 63. — COLORLESS BLOOD-CELLS OF MAN. *c*. Cell with neutrophile granules. ×600. Techn. No. 42.

The size and properties of the nucleus and protoplasm have led to the following classification:

1. The smallest leucocytes, measuring from 4 to 7.5 $\mu$. They possess a relatively large round nucleus surrounded by a narrow zone of protoplasm, so small in amount that it can scarcely be demonstrated by the usual methods (Fig. 63, *a*). These are regarded as young forms; they exhibit little activity and are chiefly found in adenoid tissue.

2. Those of the second variety have a diameter of from 7.5 to 10 $\mu$; the nucleus is spherical or deeply cleft—lobulated—and surrounded by a larger amount of granular protoplasm (Fig. 63, *b*). Occasionally several disjoined nuclei are present; the slender filaments uniting the several parts of the lobulated nucleus are frequently overlooked, which then simulates several separate nuclei. This form is very active; the lobulation of the nucleus is in fact regarded as the expression of this activity; 77 per cent. of the leucocytes of the blood are of this form.

3. The leucocytes of the third class have a diameter of from 8 to 14 $\mu$ and are characterized by their granular protoplasm; the granules are variable in quantity and react differently to stains. According to their affinity for acid, basic, or neutral dyes, oxyphile, basophile, and neutrophile

leucocytes are distinguished. The granules are probably the optical expression of metabolic processes and of phases of progressive development (see further, Techn. No. 42).

The determination of the proportionate number of, as well as the ratio between, the colored and colorless blood-corpuscles is coupled with considerable difficulty and only approximately-correct estimates can be given. In man one cubic millimeter of blood contains about 5,000,000 colored corpuscles. The white blood-corpuscles are present in the blood in much smaller number, about one in from 300 to 500 colored blood-corpuscles.

The *blood-platelets* are very unstable, colorless, round or oval discs having a diameter from one-third to one-fourth less than that of the colored blood-cells; at times they are present in the blood in large numbers.* A leading rôle in the process of coagulation of the blood is

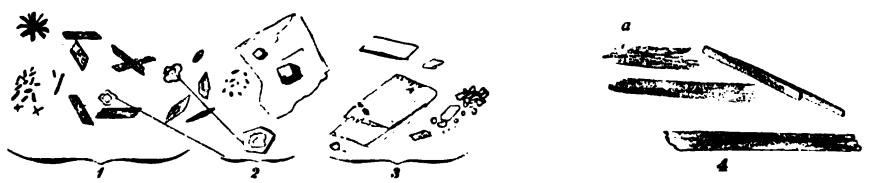


FIG. 64.—1. Hemin crystals of man; whetstone forms on the right. 2. Crystals of common salt. 3. Hematoidin crystals of man, magnified 560 times. 4. Hemoglobin crystals of the dog, magnified 100 times; *a*, a crystal falling apart lengthwise. Techn. No. 47.

ascribed to them. In animals that have nucleated colored blood-corpuscles the blood-platelets also possess nuclei.

The *elementary granules* are for the most part fatty granules transferred from the chyle to the blood. They are frequently observed in the blood of the lower mammals, but are not normally present in the blood of man.

After death, or as a result of changes within the vessel-walls, the blood under the influence of two substances which pass into solution in the plasma, fibrinoplastin and fibrinogen, coagulates, and fibrin is formed. The coagulated blood separates into two parts, the clot and the serum. The clot is red and contains all the colored blood-corpuscles, the majority of the colorless blood-corpuscles, and the fibrin, which microscopically consists of a feltwork of fine, straight, interlacing filaments. Chemically fibrin resembles glutinous connective tissue. The supernatant serum is colorless and contains a few colorless blood-cells.

* In 1 c.c. of human blood there are said to be 200,000 blood-platelets, a number that probably is below the truth, since in the methods of estimating some blood-platelets always adhere to the walls of the pipet.

The coloring substance contained in the colored corpuscles, the *hemoglobin*, possess the property of crystallizing under certain conditions and in nearly all vertebrates the crystals belong to the rhombic system. Their form in the different animals varies greatly; in man it is usually prismatic. Hemoglobin is readily decomposed. One of the decomposition products is hematin, which yields hematoidin and hemin. Crystals of hematoidin occur within the body in old extravasated blood, for example, in the corpus luteum, and are rhombic prisms of orange-red color. The hemin crystals, when well developed, are rhombic plates or needles of a mahogany-brown color; often they are very irregular in form. As a positive indication of the presence of blood they have a legal relation of great importance (see Techn. No. 47 *a*).

*Development of Colored Corpuscles.*—From the earliest period of embryonic development and during the whole of life nucleated colored blood-cells (hematoblasts, erythroblasts) are found in certain localities (see bone-marrow). Their number fluctuates and runs parallel with the energy of the blood-forming processes. By indirect division they give rise to the nonnucleated colored blood-corpuscles, that at first contain a nucleus, but subsequently lose it. As centers for the formation of blood in the embryo the liver and later the spleen, in the adult exclusively the bone-marrow, may be mentioned.

## 2. THE LYMPHATIC SYSTEM.

### THE LYMPH-VESSELS.

The walls of the larger lymph-vessels, from 0.8 to 0.2 mm. in thickness and upward, like the blood-vessels, are composed of three coats. The intima consists of endothelial cells and a network of delicate elastic fibers with elongated meshes. The media is formed of circularly-disposed smooth muscle-fibers and a few elastic fibers. The externa consists of longitudinally-arranged bundles of connective tissue, elastic fibers, and bundles of smooth muscle-fibers likewise disposed in a longitudinal direction. The walls of the smallest lymph-vessels and of the lymph-capillaries are composed exclusively of extremely delicate endothelial cells, that often have sinuous outlines. The lymph-capillaries are wider than the blood-capillaries, at frequent intervals present constrictions and dilatations, and where they branch are often considerably expanded; the networks they form are more irregular.

The question of the origin of the lymph-vessels is not yet satisfac-

torily decided; while some authors are of the opinion that the lymph-capillaries form a closed system, according to another widely-entertained view the lymph-capillaries are open toward the periphery and in direct connection with the system of intercommunicating cell-spaces of connective-tissue (juice-canal system, p. 86). These interfascicular clefts are by some set apart, as "lymph-canaliculi," from the lymph-vessels with well-defined walls composed of continuous layers of cells; other authors include the lymph-canaliculi among the lymph-vessels.

According to the first opinion the nutritive fluids (tissue-juices) diffused through the walls of the blood-capillaries that are not used in the nutrition of the tissues penetrate the closed lymph-capillaries by endosmosis; according to the second view the tissue-juices pass directly from the tissue into the patent orifices of the lymph-capillaries.

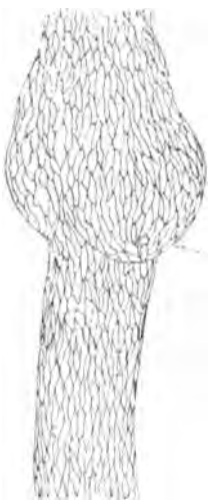


FIG. 65.—LYMPHATIC VESSEL OF THE MESENTERY OF RABBIT, showing the boundaries of the endothelial cells. × 50. Techn. No. 48.

It is a significant fact that the lymph-vessels of the pleura and of the peritoneum are in open communication with their respective cavities through small openings, the *stomata*, between the endothelial cells, which in the pleura are found at the intercostal spaces and in the peritoneum on the central tendon of the diaphragm.

### THE LYMPH-GLANDS.

The lymph-glands (lympho-glandulæ, lymph-nodes) are macroscopic bodies intercalated in the course of the lymph-vessels. Usually they are rounded oval, or flat kidney-shaped structures and differ greatly in size. At one side there is often a scar-like depression, the *hilus*, at which the efferent lymph-vessels emerge. The afferent lymph-vessels penetrate the gland at various points. Their construction becomes intelligible if we proceed from the following conception: In certain localities three to six lymph-vessels divide repeatedly, the branches anastomose, then reunite into the same or a less number of usually narrower lymph-vessels. In this way a kind of rete mirabile * is formed. The dividing lymph-vessels are

* Retia mirabilia were first described in connection with the blood-vessels. They occur along the course of both arteries and veins; the vessel suddenly breaks up into branches and these into capillaries, which reunite into a single vessel. Exquisite examples of such networks occur as the glomeruli of the kidneys.

called afferent vessels (vasa afferentia), the reuniting, efferent vessels (vasa efferentia). Within the meshes of this reticulum lie spherical and elongated masses that consist of adenoid tissue. The spherical masses, the *secondary nodules* (follicles), occupy the periphery ; the elongated masses, the *medullary cords*, the center of the lymph-gland.

The lymph-gland is enveloped in a capsule of fibrous connective tissue, which sends processes into the interior of the organ, the *trabeculæ* (Fig. 66). Finer extensions from the trabeculæ, in the form of reticular connective tissue, pierce the walls of the lymph-vessels, penetrate the secondary nodules and the medullary cords, and form a support for the numerous leucocytes present.

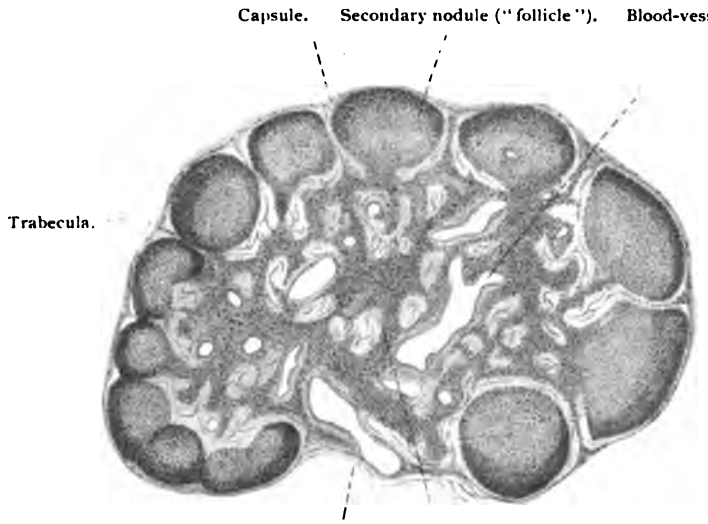


FIG. 66.—SECTION OF LYMPH-GLAND OF RABBIT. × 28. (Schaper.) Techn. No. 50.

The lymph-glands consequently consist of a *cortical* and *medullary* substance, the relative proportions of which vary greatly. The cortex contains the secondary nodules, which continue centralward directly into the medullary cords (Fig. 66). The secondary nodules and medullary cords are surrounded by the sinus-like continuations of the afferent lymph-vessels. The latter here are greatly expanded and are termed *lymph-sinuses ;* they are pierced by the connective-tissue reticulum. The lymph-vessels never penetrate the interior of the secondary nodules. The secondary nodules and medullary cords are composed of *adenoid* tissue ; that is, of reticular connective tissue, the meshes of which are crowded with leucocytes. In many of the secondary nodules there is a light, spherical area, the *germinal center*, in which karyokinetic figures

are always to be found. Multiplication of cells also occurs in the medullary cords, but in a much slighter degree. The secondary nodules are centers for the formation of leucocytes, which pass into the lymph-sinuses and thence into the vasa efferentia.

The capsule consists of fibrous connective tissue and smooth muscle-fibers, which in the large lymph-glands of some animals are arranged in long strands. The trabeculæ have the same structure; they pass between the secondary nodes and medullary cords, but do not come into contact with them, being separated from them by the lymph-sinuses. The walls of the lymph-sinuses are formed of a simple layer of plate-like cells; similar cells clothe the surface of the secondary nodules and medullary cords, and are also applied to the trabeculæ and the connective-tissue reticulum.

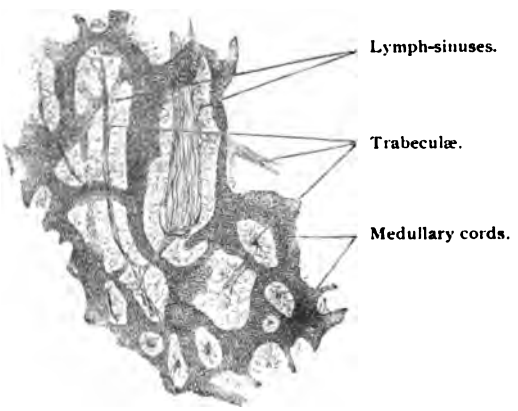


FIG. 67.—FROM A SECTION THROUGH THE MEDULLA OF A LYMPHATIC NODULE OF OX. × 51. In the upper half the trabeculæ and medullary cords are cut lengthwise, in the lower half crosswise. Both form an anastomosing network. In the lymph-sinuses the fine fibers of the reticular connective tissue are seen, which still contains leucocytes. Drawn with change of focus. Techn. No. 51.

The structure of the lymph-glands is difficult to recognize, owing to several complications. These consist in: 1, the merging of neighboring secondary nodules; 2, the anastomosis of the medullary cords in the form of a coarse network; 3, the network formed by the trabeculæ; 4, the interlacing of the networks formed by the medullary cords and the trabeculæ; 5, the presence of leucocytes in the lymph-sinuses, which must be removed by special methods. The secondary nodules, the medullary cords, and the leucocytes in the lymph-sinuses form a soft mass, that has been named the *pulp* or parenchyma of the lymph-gland.

The majority of the blood-vessels enter at the hilus, the others at various points on the surface of the gland. The latter are smaller vessels and divide in the capsule and in the large trabeculæ, in the axes

of which they run. The large artery entering at the hilus divides into a number of branches, that are surrounded by richly-developed connective tissue. The branches are principally distributed to the adenoid tissue, only a few entering the trabeculæ; they pass through the lymph-sinuses, into the medullary cords, then into the secondary nodules, and in both situations break up into rich capillary networks which supply the oxygen needed in the formation of the leucocytes. The veins emerge at the hilus.

The nerves are few in number, the supply including bundles containing medullated and nonmedullated fibers; their ultimate distribution is still undetermined.

## The Peripheral Lymph-nodules.

(Noduli Lymphatici.)

Adenoid tissue is not confined to the lymph-glands; it occurs in many mucous membranes, in different degrees of development, sometimes as *diffuse*, sometimes as *definitely circumscribed* infiltrations of leucocytes. These formations are not included in the lymphatic system. More highly-specialized structures, nodules with germinal centers, closely resembling the secondary nodules of the lymph-glands, are also found in the mucous membranes; these are named *peripheral lymph-nodes* and are included in the lymphatic system. In many mucous membranes they occur isolated, as the *solitary nodules* (solitary follicles), or grouped, as the agminated nodules (Peyer's patches), and always lie in a simple layer in the tunica propria close beneath the epithelium (see the digestive organs). The number and distribution of the peripheral lymph-nodes are subject to considerable fluctuation, not only in the different species of animals, but in different individuals; since their mass varies and there are frequent transitions to circumscribed and to diffuse infiltrations, it is probable that they are temporary structures that arise and disappear during life. They are distinguished from the real lymph-glands, above all by their less intimate relation to the lymph-vessels, which do not form an encircling sinus for the follicle.* But the possession of a germinal center, a brooding-place for young leucocytes, appears in so far to entitle them to a place in the lymphatic system. The young leucocytes only in part enter the lymph-vessels; many wander through the epithelium to the surface of the mucous membrane.

* The only exceptions occur in the rabbit, in which the sinus is present in the Peyer's patches, but not in the solitary follicles.

## The Lymph.

The lymph is a colorless fluid in which leucocytes (lymph-corpuscles) and granules are suspended. The latter are immeasurably small, consist of fat, and are principally found in the lymph- (or chyle) vessels (lacteals) of the intestine; frequently they are present in enormous numbers and then they impart the white color to the chyle. In other lymph-vessels the fatty granules occur sparingly.

## The Spleen.

The spleen is a "blood-vessel gland" and consists of a connective-tissue *capsule* and a soft red mass, composed of blood-vessels and adenoid tissue, the *spleen-pulp*.

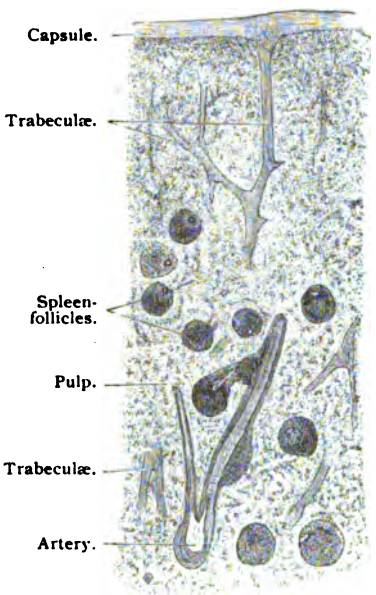


Fig. 68.—From a Cross-section of Human Spleen, showing well-developed spleen-follicles, each pierced eccentrically by an artery. The right branch of the artery has a continuous sheath of adenoid tissue. × 10. Techn. No. 53.

The *capsule* is invested by a reflection of the peritoneum, with which it is firmly united, and is composed of dense fibrous connective tissue, smooth muscle-fibers, and a network of elastic fibers. Numerous cylindrical or band-like prolongations, the *trabeculæ*, extend into the interior of the organ, where they form a framework in the spaces of which lies the spleen-pulp. The trabeculæ also contain smooth muscle-fibers. At the hilum of the spleen the capsule furnishes special sheaths for the blood-vessels—adventitial sheaths—which blend with the externa and accompany them for long distances. The sheaths of the arteries are the seat of numerous leucocytes, that in the form of a continuous envelope accompany the vessel in its entire course (as in the guinea-pig), or that, as in man, the cat, etc., are confined to certain localities, where they form spherical masses, from 0.2 to 0.7 mm. in size, the so-called spleen-follicles (*Malpighian corpuscles*). Between these many intermediate forms exist, as in the mouse and rabbit.

The *spleen-follicles* are usually situated in the forks of the smaller

arteries, and in such a manner that the artery pierces the center or the side of the follicle. In their minute structure they entirely agree with the secondary nodules of the lymph-glands, and even occasionally

FIG. 69.—ELEMENTS OF HUMAN SPLEEN. × 560. 1. Colorless blood-cells. 2. Epithelial cells. 3. Colored blood-corpuscles. 4. Cells containing granules; the upper one enclosing a blood-corpuscle, *b*. Techn. No. 52.

FIG. 70.—RETICULAR CONNECTIVE TISSUE OF HUMAN SPLEEN. × 560. Drawn from the edge of a shaken preparation. Techn. No. 53 *a*.

FIG. 71.—THREE KARYOMITOTIC FIGURES FROM A SECTION OF SPLEEN OF DOG. × 560 The filaments are not visible with this magnification. Techn. No. 54.

contain germinal centers. The spleen-follicles are also temporary structures; continually some are undergoing regressive change and new ones are developing.

The *spleen-pulp* forms a network of cords, which, similar to those

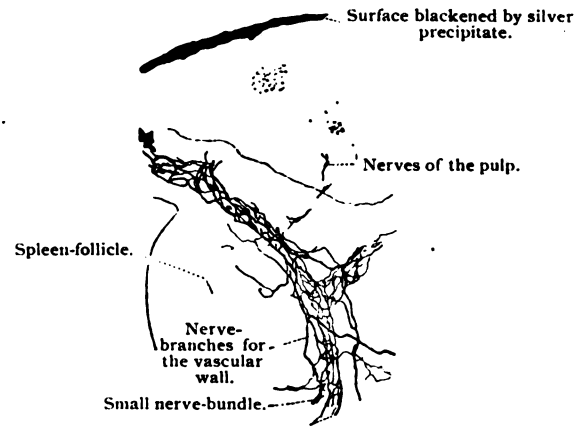


FIG. 72.—SECTION OF SPLEEN OF MOUSE, MAGNIFIED 85 TIMES, showing the nerves supplying the wall of an artery. The boundary between the spleen-pulp and the artery, the sheath of which is infiltrated in its entire length with leucocytes, is indicated by a dotted line. Techn. No. 56.

of the lymph-glands, occupy the interstices of the trabecular framework. Occasionally the cords are connected with the spleen-follicles. The spleen-pulp is composed of a delicate connective-tissue reticulum

and numerous cellular elements. The latter are in part leucocytes, in

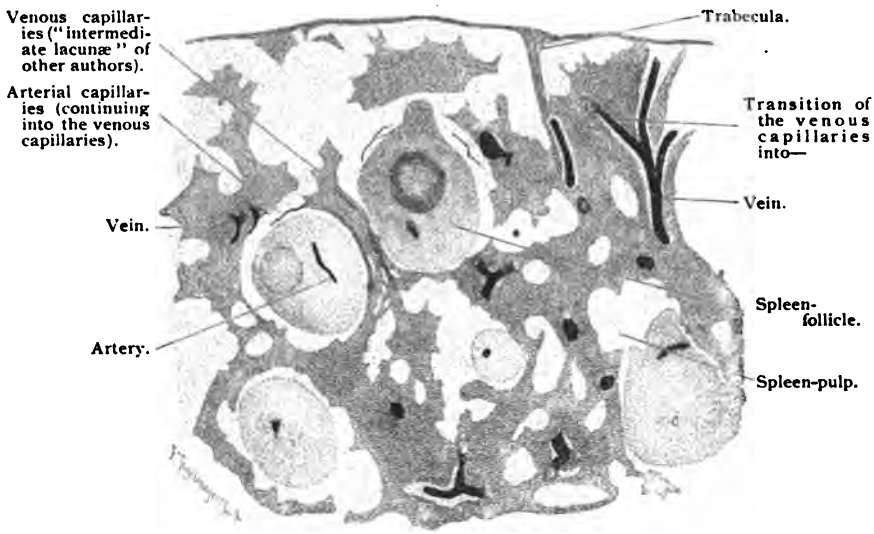


FIG. 73, A.—SECTION THROUGH AN INJECTED SPLEEN OF CAT. Techn. No. 57.

part slightly larger nucleated cells, also cells containing colored blood-corpuscles and free colored blood-corpuscles. A granular pigment is present.

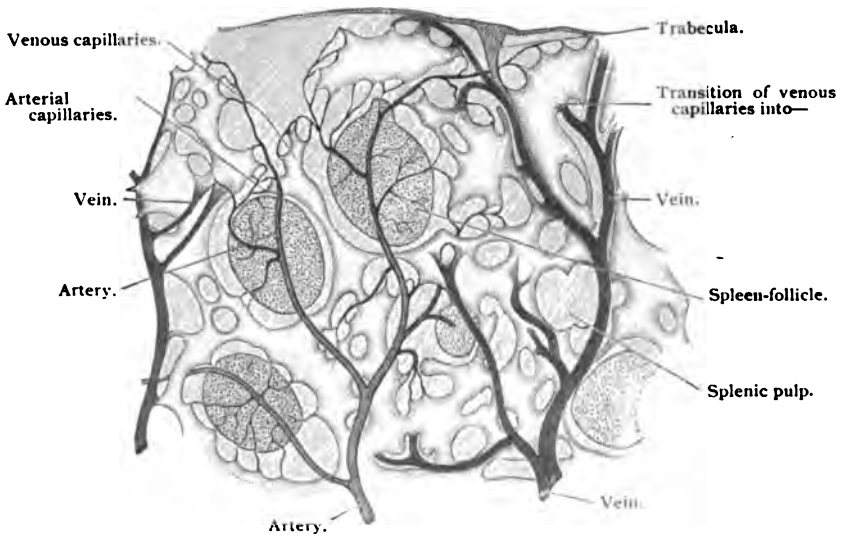


FIG. 73, B.—SCHEMATIC DRAWING OF SECTION 73, A.

*The Blood-vessels.*—The arteries of the spleen give off branches to the

trabeculæ and to the pulp-cords and supply the dense capillary network of the spleen-follicles. There are no anastomoses between the arteries. The thin-walled veins proceed from a wide-meshed network of capillaries (venous spaces, venous capillaries) occupying the intervals between the trabeculæ and the pulp-cords (Fig. 73). The medium-sized and larger veins run alongside the arteries and frequently lie in furrow-like depressions in the trabeculæ. The precise mode of communication between the arteries and the veins is not yet satisfactorily determined. The arteries break up into slender capillaries which do not anastomose with one another.* According to one view, the arterial capillaries are directly continuous with the "venous" capillaries and the vascular channels of the spleen are closed on all sides. Other authors hold that the arterial capillaries pass into spaces without definite walls, "intermediate lacunæ," which connect with veins with perforated, sieve-like coats, and that the latter establish the communication with the veins with closed walls.

The superficial *lymphatics* on the surface of the spleen, numerous in the lower mammals, are scantily developed in man. The deep lymphatics in the interior of the spleen are also few in number; the exact relations of the latter have not yet been fully investigated.

The *nerves*, consisting of a few medullated fibers and many naked axis-cylinders, follow the course of the trunks and branches of the arteries, supply the muscle-fibers of the latter and of the trabeculæ (Fig. 72). Plexuses of nonmedullated nerve-fibers occur in the spleen-pulp, that probably proceed from the branches of the medullated nerve-fibers just mentioned, and are in part sensory in their nature.

## TECHNIC.

No. 35.—*The Heart and the Large Blood-vessels.*—Cut out a papillary muscle from a human heart, a piece of the aorta 2 cm. long, a piece 1 or 2 cm. long of the bronchial artery with its veins and the surrounding connective tissue, a piece of the renal vein 1 cm. long, and suspend them on a thread in a bottle containing 40 c.c. of absolute alcohol. After twenty-four or forty-eight hours the objects are ready to section. Embed them in liver (the artery and vein may be embedded together and will not be injured by strong compression), cut thin cross-sections, stain them in Hansen's hematoxylin, two to five minutes (p. 36), and mount in damar (Fig. 53, 56, 57, 58). The elastic fibers do not stain, but with the high power can be often distinctly recognized.

* In injected and macerated spleens the pulp can be washed out, and then the slender terminal branches of the arteries can be seen lying together in a leash or pencil.

The arrangement of the elements of the externa cannot be satisfactorily appreciated in cross-sections, often all appear to be circularly disposed (a portion of them are circularly arranged—for example, those of the innermost strata of the external elastic membrane). The exact arrangement can only be seen in longitudinal sections, which also show the muscle-fibers of the adventitia plainly.

No. 36.—*Elastic Fibers of the Blood-vessels.*—Stain objects fixed in absolute alcohol, according to No. 35, with orcein (No. 11, p. 40) and preserve in damar-varnish (Fig. 59).

No. 37.—*Small Blood-vessels and Capillaries.*—From the base of a human brain slowly strip off pieces of the pia 1 to 3 cm. in length (in this way delicate blood-vessels that penetrate the brain vertically are withdrawn), shake them in distilled water to free them from adherent fragments of brain-tissue, and place them in 50 c.c. of Zenker's fluid (p. 31) for one hour; transfer them for from one to three hours to water (for one hour to running water), and harden them in about 40 c.c. of gradually-strengthened alcohol (p. 33). Examine one of these pieces in a watch-glass on a black background and it will be seen that small vessels are isolated.

*a.* With fine scissors cut off small twigs with their ramifications, stain them for from two to five minutes in Hansen's hematoxylin (p. 36) and mount in damar (Fig. 54).

*b.* From the larger twigs of the cerebral blood-vessels cut pieces about 5 mm. long, slit them open lengthwise, stain them in Hansen's hematoxylin, and place them on a slide with the adventitia side down. Mount in damar. By changing the focus the three coats of the vessels and their general arrangement can be seen.

Capillaries may be found on examining fresh brain-tissue. They are recognized by their parallel outlines and the oval nuclei of their endothelial cells; they are also found in other preparations, for example in Techn. No. 9.

No. 38.—*Epithelium* (*Endothelium*) *of the Blood-vessels.*—Decapitate a rabbit, open the abdomen by a crucial cut made with the scissors; insert a cork frame about 2 cm. square under the mesentery, span the membrane smoothly and fasten it with quills or hedgehog spines, taking care to touch it as little as possible. Cut it off all around the frame and place the stretched membrane with the frame in 20 or 30 c.c. of 1 per cent. silver solution. In about thirty seconds the solution becomes turbid and milky; remove the frame, carefully wash the membrane with distilled water, place the whole in a white capsule containing 100 c.c. of distilled water and expose it to direct sunlight. In a few minutes a brown coloration appears. Now transfer the whole to 50 c.c. of 70 per cent. alcohol (the membrane must be submerged in the alcohol); in a half-hour cut out small pieces 5 or 10 mm. long and mount them in damar. In the absence of sunlight, take the preparation from the silver solution, wash it, place it for about twenty hours in about 30 c.c. of 70 per cent. alcohol, then in alike quantity of 90 per cent. alcohol, and expose it to

sunlight on the first opportunity. It must not be forgotten that the whole blood-vessel and not a section of it is present, so that in order to obtain a view such as that in Fig. 55 the surface of the vessel must be in focus.

No. 39.—*Elastic Fenestrated Membranes.*—See Techn. No. 14.

No. 40.—*Development of Capillaries.*—Chloroform a seven-day-old rabbit, fasten it with pins on a cork-plate, open the abdomen by a crucial incision, quickly remove the spleen, stomach, and attached greater omentum and place these parts in 80 c.c. of a saturated aqueous solution of picric acid (p. 21). In this solution the omentum, otherwise difficult to separate, spreads out easily. After one hour cut it off, transfer it to 60 c.c. of distilled water, and divide it with the scissors into pieces about 1 cm. square. Place such a piece on a dry slide, remove the water with filter-paper, and with needles spread it out as smooth as possible, which is the more easily done the less moisture there is present. Put one or two drops of Hansen's hematoxylin on the preparation. In from one to five minutes drain off the hematoxylin and place the slide with the preparation in a flat dish containing distilled water ; the membrane will soon float from the slide, but will remain smooth, and in five minutes should be transferred with the section-lifter to a watch-glass containing eosin (p. 37), in which it should remain three minutes. It should then be washed for one minute in distilled water and placed on a slide ; the water should be absorbed with filter-paper, any wrinkles smoothed out with needles, and a cover-glass with a drop of dilute glycerol suspended from its lower surface applied. The preparation may be mounted in damar instead of glycerol (that is, dehydrated in 95 per cent. alcohol, cleared in oil of bergamot, and then mounted in damar), but the finer structural details are apt to be lost. The colored blood-corpuscles are stained a bright red by the eosin (Fig. 61).

In spreading the membrane on the slide, delicate young capillaries may be easily torn from the older capillaries and then simulate " isolated cells containing blood-corpuscles ;" such artificial products have been described as " vasoformative cells."

No. 41.—*Colored Blood-corpuscles of Man.*—Carefully cleanse a slide and a small cover-glass (finally with alcohol). With a clean needle prick the finger-tip at one side ; lightly touch the first drop of blood that exudes with the cover-glass, and without the addition of any reagent immediately place it on the slide. With the high power many colored corpuscles adhering to one another by their broad surfaces, forming the so-called rouleaux, may be seen, as well as isolated colored and colorless blood-corpuscles. The distortion of many of the colored corpuscles is due to evaporation, in consequence of which they are beset with minute spines, are *crenated.* If a drop of water be placed at the edge of the cover-glass, the corpuscles soon become decolorized and the water acquires a yellowish tinge ; the corpuscles then become spherical, have the appearance of pale circles, and finally disappear. The student is advised to study the decolorization of a single corpuscle. In Fig. 62, 6,

the tinged area surrounding the bleached corpuscles is somewhat too deeply shaded.

No. 42.—*Permanent preparations of colored and colorless blood-corpuscles* are made by Ehrlich's dry method. The method accurately carried out, after some practice, yields good results, but with unskilful manipulation many caricatures arise and mislead the inexperienced. The employment of this method for purposes of investigation and discovery requires great skill and great caution in judgment.

*Preliminary Manipulations.*—For each preparation two *thin* cover-glasses are required (they must not be over 0.1 mm. thick). Place them for a few minutes in dilute hydrochloric acid, then in distilled water, and finally in alcohol. It is best to take cover-glasses that have never been used. Prepare a mixture of equal parts of absolute alcohol and ether (about 5 c.c. of each). Cleanse the tip of the finger first with soap and water, then with a tuft of clean cotton-wool moistened in the alcohol-ether mixture. With a clean needle (not previously used for anatomic purposes) prick the pad of the finger that has been made slightly hyperemic by compression; take up a cover-glass with the forceps (not with the fingers), press it lightly upon the blood that exudes and place it on the second cover-glass, with one edge projecting slightly. The drop of blood will spread out in a thin film between the two glasses, which are then *slipped* apart by means of two forceps. By this manipulation the influence of the insensible perspiration on the blood-corpuscles is prevented, which otherwise would shrink or lose their hemoglobin.

Exposed to the air, the blood on the cover-glasses dries in a few minutes; they are then to be placed in the alcohol-ether mixture for fixation. In from one-quarter to two hours they should be removed, again dried in the air, when they are ready for further treatment, which may be applied immediately or later, since the preparations thus "fixed" may be preserved for a long time.

*a. Oxyphile* (*Eosinophile*, *a*) *Granules.*—Place the cover-glass preparations for twenty-four hours in about 4 c.c. of distilled water, to which about 10 drops of eosin solution have been added. Rinse one minute in distilled water and stain from one to five minutes in a watch-glass with Hansen's hematoxylin (p. 36). Transfer to distilled water; remove in five minutes and let the preparations dry in air under a bell-glass. Mount in damar. The colored blood-corpuscles and the oxyphile granules of the colorless corpuscles are stained a bright red, the nuclei are blue. The oxyphile granules occur in the leucocytes of normal blood, of lymph, and of the tissues, but are uncommon in normal blood. A magnification of 400 diameters is sufficient to find them.

*b. Basophile Granules.*—Two groups are distinguished, the $\gamma$-granules and the $\delta$-granules. The *$\gamma$-granules* (mast-cell granules), which occur only in the leucocytes of pathologic blood, are stained according to the method given in No. 6. When the staining is completed, proceed as in *a*. The blue-violet granules are coarser than the—

*$\delta$-granules*, which occur in the round nucleated leucocytes of normal and other blood. Stain the cover-glass preparations from five to ten

minutes in 5 c.c. of methylene-blue solution (p. 25), wash, dry, and mount in damar. These granules are minute and scarcely to be seen with the usual high-power dry lenses; an immersion lens should be used. In staining with methylene-blue not infrequently the film of blood floats from the cover-glass; this may be prevented by passing the dry cover-glass preparation rapidly through a flame before staining.

*c. Neutrophile* (ε-) *Granules.*—Dissolve (1) 1 gm. of orange-yellow extra in 50 c.c. of distilled water; (2) 1 gm. of acid fuchsin extra in 50 c.c. of distilled water; (3) 1 gm. of crystalline methyl-green in 50 c.c. of distilled water, and let the three solutions settle. Then mix 11 c.c. of solution (1) with 10 c.c. of solution (2), and add 20 c.c. of distilled water and 10 c.c. of absolute alcohol; to this mixture add a mixture of 13 c.c. of solution (3), 10 c.c. of distilled water, and 3 c.c. of absolute alcohol. The whole is then allowed to stand for one or two weeks. In this "triacid solution" the cover-glass should be placed for fifteen minutes, then washed, dried, and mounted in damar. The neutrophile granules, which are found in leucocytes with lobulated nuclei, in normal and other blood, are of a violet color, and are easily seen with the usual dry high-power lenses; the oxyphile granules and the colored blood-corpuscles are of a yellow-brown or chocolate-brown color, the nuclei a bright blue-green, though their outlines are not so distinct as in the hematoxylin preparation.

No. 43.—*Blood-platelets.*—Mix about 5 drops of an aqueous solution of methyl-violet (p. 25) with about 5 c.c. of salt solution (p. 19). **Filter** the mixture and place a drop of it on the tip of the finger; prick the finger through the drop; the blood as it exudes mixes with the methyl-violet; take up a drop with the cover-glass and examine with the high power. The platelets are stained an intense blue, have a peculiar luster, are disc-shaped, and should not be confused with the white blood-corpuscles likewise stained blue (Fig. 62, 9). They are numerically variable elements, occurring in large numbers in the blood of one individual, while in the blood of another they are only to be found singly here and there. Care must be taken not to confuse them with foreign particles, which may occur even in the filtered staining solution.

No. 44.—*Colored Blood-corpuscles of the Frog.*—Prepare the slide and treat the blood like No. 41.

No. 45.—*For Legal Purposes.*—Since it is usually dried blood that is to be examined, dissolve small particles of dried blood in 35 per cent. potash solution on a slide; blood-stained pieces of linen may be teased in a drop of the same solution. Although the colored blood-corpuscles of domestic mammalian animals are smaller than those of man, it is nevertheless impossible from the size of the blood-cell to determine its source. On the other hand, it is easy to distinguish the disc-shaped corpuscles of mammals from the oval elements of other vertebrates.

No. 46.—*Colorless Blood-corpuscles* (*Leucocytes*) *in Motion.*—*Preliminary manipulations:* Carefully cleanse a slide and cover-glass with

alcohol. Kill a frog, grasp it by its hind legs, dry its back somewhat with a cloth, and with fine scissors make an incision 1 cm. long parallel to and close beside the vertebral column. Introduce a capillary pipet into the wound (with the tip directed forward) and suck the tip full. A small drop is sufficient; blow it on to the slide, cover it quickly, and seal the edges with melted paraffin (p. 48). Such a preparation shows colored and colorless blood-cells; at first the nuclei of the former are indistinct. The nuclei of living blood-corpuscles are in general not to be seen. For the study of ameboid movement, select leucocytes the protoplasm of which is partly granular and that are not spherical. The movements are slow; of this one may convince one's self by studying a single leucocyte and making sketches of it at intervals of from one to two minutes. Study with the high power (Fig. 4).

No. 47.—*Blood-crystals.*—*a. Hemin crystals* are easily obtained. Cut a small strip, about 3 mm. long, from a piece of linen previously saturated with blood and dried and place it with a pinhead-sized crystal of common salt on a clean slide; add a large drop of glacial acetic acid and with a glass-rod stir the linen and salt for about one minute, until the acid acquires a brownish tinge. Then heat the slide over the flame until the acetic acid is evaporated. Remove the linen and examine the dry brown places on the slide with the high power (from 240 diameters up). Occasionally the *brown crystals* may be seen without the cover-glass and without a mounting medium, lying next to numerous fragments of white salt-crystals (Fig. 64, 1). To preserve, add a large drop of damar and apply a cover-glass. The hemin crystals differ greatly in form and size. In the same slide well-developed crystals lying singly, crosswise over one another, or in stellate groups are seen, with whetstone shapes and minute particles that scarcely exhibit crystallization. The demonstration of the hemin crystals is of great importance in forensic cases. While it is easy to obtain the crystals in large stains on wearing apparel, it is difficult, when the stains are small, especially on rusty iron, to prove that they are from blood. The instruments and reagents employed in such investigations must be absolutely free from contamination.

*b. Hematoidin crystals* are obtained by teasing old blood extravasations; they can be recognized macroscopically by their reddish-brown color—for example, in the corpus luteum, in cerebral hemorrhages.

*c. Hemoglobin crystals* are obtained by transferring 5 c.c. of the blood of a dog to a test-tube, adding a couple of drops of ether, and shaking vigorously until the blood becomes lake-colored. Then spread a drop on a slide and let the preparation dry in the cold. When crystallization has occurred, add a drop of glycerol and apply a cover-glass. The large crystals often exhibit a tendency to cleave lengthwise (Fig. 64, 4 *a*).

No. 48.—*Lymph-vessels.*—For the study of the *walls* of the larger lymph-vessels select the vessels opening into the inguinal glands, that

are large enough to be taken out with forceps and scalpel. Prepare like the large blood-vessels, No. 35, or after No. 37 *b*.

No. 49.—For the representation of the more *delicate* lymph-vessels, of their course and arrangement, the method of interstitial injection is often employed. The needle of a hypodermic syringe filled with Berlin-blue is thrust haphazard into the tissue; this is a crude method, the results of which are of very doubtful value. Even though here and there actual lymph-vessels may thus be filled, in most cases the injection-mass is simply driven forcibly into the interfascicular clefts of the connective tissue. The value of any decision with regard to "radicles of lymph-vessels" and to "lymph-spaces" thus exhibited may be inferred.

No. 50.—*Lymph-glands.*—For a general view the mesenteric glands of kittens and young rabbits are suitable. For fixation and hardening place them in 30 c.c. of absolute alcohol; in three days thin sections can be readily made and should be taken so that they pass through the hilus, which is easily recognized macroscopically by an external depression. Longitudinal sections passing through the poles of the gland are best, though transverse sections are also useful. Stain six or eight sections in Hansen's hematoxylin for from two to three minutes, then in eosin, at the most one minute (p. 37, 3 *b*), transfer them to a test-tube half filled with distilled water and shake them for from three to five minutes. Pour the shaken sections into a flat dish; the cortex and medulla can be macroscopically distinguished by the uniformly blue color of the former and the variegated appearance of the latter. Mount in damar. With the lower power, fields similar to that of Fig. 66 may be seen in favorable sections. The trabeculæ are but slightly developed. The adipose tissue adhering to the glands must not be taken for reticular tissue. High magnification is of no advantage; the sharp outlines disappear and the picture loses in distinctness.

No. 51.—*Lymph-glands of mature animals and of man* are difficult to understand, because the entire cortex is transformed into a continuous mass sprinkled with irregular germinal centers. In shaking the sections the germinal centers are apt to fall out and leave round spaces macroscopically recognizable. The lymph-sinuses can be only indistinctly made out. The mesenteric follicles of the ox are well adapted for the representation of the network of the *medullary cords* and *trabeculæ*. Place pieces 2 cm. long in 200 c.c. of concentrated aqueous picric-acid solution, and after twenty-four hours, with a sharp knife moistened with water, endeaver to cut thin sections. This is not so easily done as after alcohol fixation, but slightly thicker sections can be used. Place the sections for one hour in 100 c.c. of distilled water, which must be changed frequently, then stain with Hansen's hematoxylin and eosin and shake them (see No. 50). Mount in damar (p. 45). The trabeculæ are red, the medullary cords blue; with low magnification the appearance of the section is like Fig. 67; with high magnification the reticular connective tissue of the lymph-sinuses can be seen; the majority of the leucocytes

occupying the meshes become loosened by the treatment with picric acid and are lost in the shaking.

No. 52.—*Elements of the Spleen.*—Make an incision through a fresh spleen; with a scalpel obliquely applied scrape the cut surface and examine a little of the red mass adhering to the blade in a drop of salt solution. Use the high power. Often, especially in animals, only colored and colorless blood-corpuscles are found; some of the latter contain minute granules. In human spleens, in addition to the numerous colored blood-corpuscles altered in form, endothelial cells of the blood-vessels are found; the latter were formerly called "spleen-fibers" (Fig. 69, 2, 3). In many human spleens, multinucleated cells containing blood-corpuscles are often sought in vain (Fig. 69, 4).

No. 53.—*The Spleen.*—Without cutting it, "fix" the entire spleen in Müller's fluid, using one liter for a human, 200 to 300 c.c. for a cat's spleen. After two weeks for the cat's, five weeks for the human spleen, wash for from one to two hours in, if possible, running water, cut out pieces 2 cm. square and harden them in 60 c.c. of gradually-strengthened alcohol (p. 33). Sections not too thin are to be stained in Hansen's hematoxylin and mounted in damar. If it is desired to stain the trabeculæ, after the staining in hematoxylin is completed place the section for one-half minute in eosin. In successful preparations the pulp and the Malpighian bodies are blue, the trabeculæ rosy, the vessels, distended with blood-corpuscles, brown. If the staining in eosin is prolonged beyond thirty seconds the blood-corpuscles become brick-red, the trabeculæ dark red, and the distinction between them is apt to be lost. The sections are most satisfactory when examined with a very low power (Fig. 68); with the high power the outlines are often indistinct. Fixation in Zenker's fluid is also recommended.

No. 53 *a.*—*Reticular Connective Tissue of the Spleen.*—Shake a thin section fixed and stained like No. 53 for about five minutes in a test-tube half filled with distilled water. Mount in glycerol. The leucocytes are difficult to dislodge; the narrow-meshed network can only be seen at the edges of the preparation (Fig. 70).

No. 54.—*Karyomitotic Figures in the Spleen and Lymph-glands.*—For this purpose small pieces (5 or 10 mm. long) of *warm living* spleen and lymph-glands should be fixed in chromic-acetic-osmic acid (p. 22), and hardened in alcohol. Stain thin sections in safranin (p. 25). Mount in damar. The karyomitotic figures of mammals are so small, that with the usual magnification (560 diameters), they can only be found by the practised microscopist. They are detected by their deep-red color (Fig. 71).

No. 55.—*Blood-vessels of the spleen* are incidentally obtained by injecting the stomach and intestine (compare with No. 110).

No. 56.—*Nerves of Spleen.*—For this purpose the spleen of the mouse is best suited. Halve it and apply Golgi's method for the demon-

stration of the elements of the nervous system (p. 41). It is sometimes sufficient to place the object in the osmio-bichromate mixture (in a warm chamber) for three days and for the same length of time in the silver solution; often a repetition of the whole process once or twice yields good results.

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## III. THE ORGANS OF THE SKELETAL SYSTEM.

The skeletal system mainly consists of a large number of hard bodies, the bones, which are joined together by special structures and form in their entirety the skeleton.

In the embryo the greater part of the skeleton consists of cartilage, which in the course of development is supplanted by bone and with the exception of a few remnants disappears; such remnants are the costal cartilages and the cartilages of the joints, which cover the articular surfaces of many bones. Skeletal cartilages are also found in the respiratory passages and in the organs of special sense.

### THE BONES.

On sawing through a fresh bone, at once it will be seen that its texture is not everywhere alike, but that the osseous tissue appears in two forms; the one, a very dense, firm, apparently structureless substance, constitutes the principal portion of the periphery and is termed *compact bone* (substantia compacta); the other, toward the axial cavity, appears as an irregular reticulum of thin osseous lamellæ and slender trabeculæ, and is called *spongy bone* (substantia spongiosa). The interstices of the spongy bone, as well as the central marrow-cavity, are filled by a soft mass, the *bone-marrow;* the surface of the bone is enveloped in a fibrous membrane, the *periosteum*. The proportion between the compact and the spongy substance is different in the *short* bones, which consist chiefly of the latter, the compact substance being limited to a narrow zone at the periphery. *Flat* bones have sometimes thicker, sometimes thinner outer shells or crusts of compact substance, while the interior is filled with spongy substance. In the epiphyses of the long bones, as in the short bones, the spongy substance preponderates.

The *spongy substance* consists entirely of osseous tissue; the *compact substance*, on the other hand, contains besides the bone canaliculi and lacunæ, a second system of coarser channels, from 22 to 110 $\mu$ wide,

which divide dichotomously and form a wide-meshed network. These channels contain the blood-vessels and are named *haversian canals.* In the long bones, in the ribs, in the clavicle, and in the inferior maxilla their course is parallel to the long axis of the bone; in short bones they run mainly in one direction, for example, vertically in the vertebræ; in the flat bones their course is parallel to the surface, not infrequently along lines that radiate from a point, as in the tuberosity of the parietal bone. The haversian canals open on the outer surface of the bone (Fig. 74, x), as well as on the inner surface (Fig. 74, xx), directed toward the substantia spongiosa.

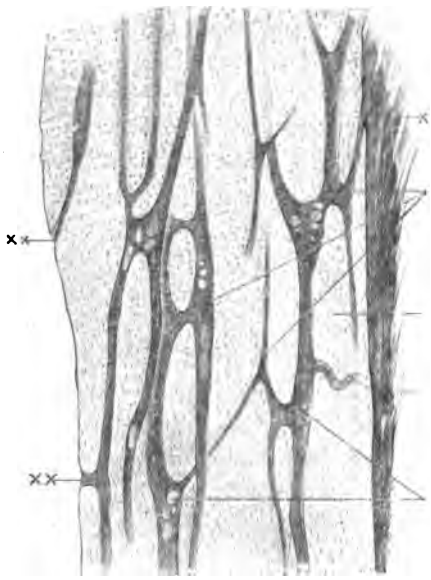


FIG. 74.—FROM A LONGITUDINAL SECTION OF A HUMAN METACARPUS. × 30. Fat-drops are seen in the Haversian canals. At x Haversian canals open on the outer, and at xx on the inner surface of the bone. Techn. No. 58.

The ground-substance of compact bone is arranged in lamellæ, that is, the osseous fibrillæ are joined in bundles, and these placed side by side form thin plates or lamellæ. According to the disposition of these plates three lamellar systems may be distinguished: an annular or *haversian system*, which in cross-sections exhibit eight to fifteen lamellæ concentrically arranged around an haversian canal; these lamellæ are called *haversian* or *special lamellæ* (Fig. 75). Between the haversian lamellar systems, that come into contact only here and there, are irregularly-disposed lamellæ, the *interstitial lamellæ;* these are connected with the third lamellar system, the *circumferential lamellæ*, in which the osse-

ous strata encircle the outer and occasionally the inner free surface of the bone. The circumferential lamellæ contain an extremely variable number of channels for blood-vessels, which, unlike the haversian canals, are not the centers of annular systems of lamellæ; they are called Volkmann's canals and the contained vessels, the "perforating vessels." The latter frequently connect with the vessels of the haversian canals; the passage of the Volkmann's canals into the latter is a very gradual one. The bone lacunæ in the compact substance have a definite position. In the haversian systems their long axis is parallel to the long axis of the

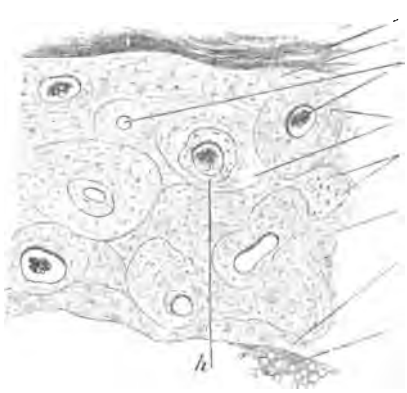


FIG. 75.—FROM A CROSS-SECTION OF A METACARPUS OF MAN. × 50. The haversian canals, *h*, still contain marrow (fat-cells). Techn. No. 58.

haversian canals and they are bent so that cut transversely in the cross-section of an haversian canal they appear concentrically curved. In the interstitial lamellæ the lacunæ are placed irregularly; in the circumferential lamellæ so that their surfaces extend parallel to the surfaces of the lamellæ. The bone canaliculi open into the haversian canals and on the free outer and inner surfaces of the bone.

The *bone-marrow* occupies the axial cavity of the tubular bones, fills the interstices of the spongy substance, and is also found in the larger haversian canals. It is of a red or yellow color and therefore two varieties are distinguished, the *red marrow* and the *yellow marrow*. The red marrow is found in the flat bones, in the vertebræ, in the base of the skull, in the sternum, in the ribs, and in all young bones (also in all the long bones of small animals); the yellow marrow occurs in the short and long bones of the extremities. In old and in sick persons the marrow is mucoid and reddish-yellow and is then called *gelatinous* bone-marrow; it is only characterized by its poverty in fat.

The elements of *red marrow* comprise a delicate connective-tissue

reticulum, a few fat-cells, larger and smaller marrow-cells,* and giant-cells (myeloplaxes) (Fig. 76). In the larger marrow-cavities the connective tissue forms a membrane, the *endosteum*, which lines the free surface. The marrow-cells exhibit manifold forms resembling leucocytes; the giant-cells are structural anomalies representing leucocytes enlarged and altered in form; they are huge, extremely irregular, uninucleated, or multinucleated masses of protoplasm. The shape of the nucleus varies

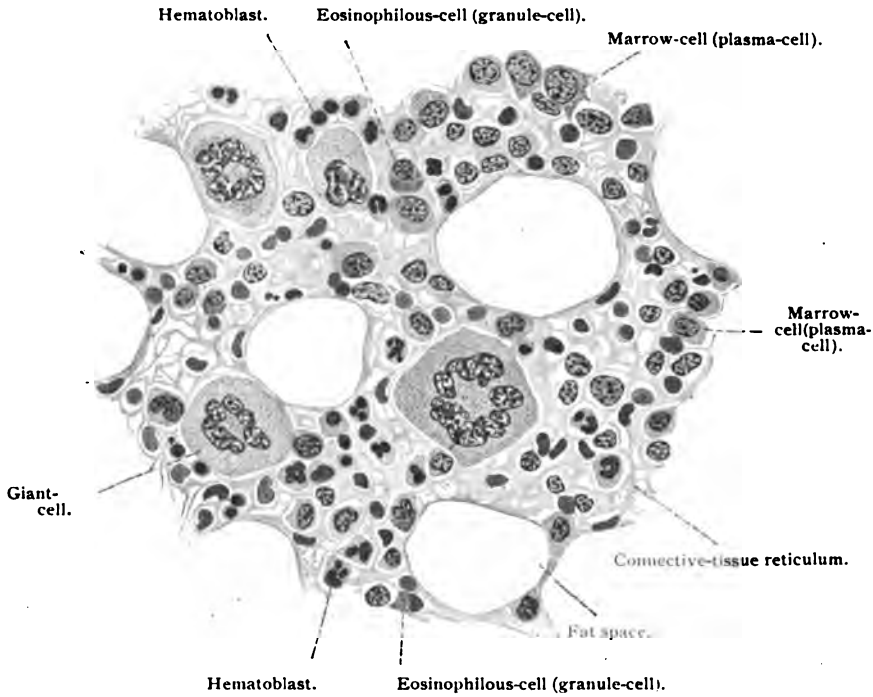


FIG. 76.—SECTION OF BONE-MARROW OF RABBIT, SHOWING THE DELICATE CONNECTIVE-TISSUE RETICULUM CONTAINING THE DIFFERENT ELEMENTS OF THE MARROW. × 400. (Schaper).

greatly; it may be round, lobulated, band- or hoop-shaped, or it may fashion a network. A uninuclear giant-cell may become multinuclear through the division of the nucleus by constriction, or a corresponding part of the protoplasm may be set free with the nucleus and the result is

* The attempt has been made to classify the cells of bone-marrow according to their source, cells with a slightly-developed cell-body (Fig. 77, 1) having been named "lymphocytes," those with a well-developed cell-body (3, 4, 5) "myelocytes." The possibility that the lymphocytes originate not only in the lymph-glands and related organs, but also in the bone-marrow, can not be excluded; likewise, the occurrence of myelocytes in the spleen and occasionally in the lymph-glands is certain. Further, if the fact that numerous intermediate forms exist is considered, the untenableness of this attempted classification is evident.

a uninuclear cell. The supposition that these processes of division indicate the phenomena of a reversed series of processes, the merging of several cells into one, has very little probability, since the process of budding has been observed in living cells. Finally there are found in the red marrow nucleated cells with yellow-colored protoplasm like that

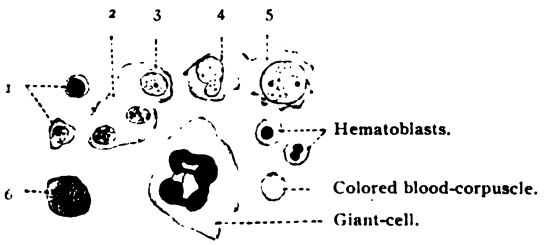


FIG. 77.—ELEMENTS OF HUMAN BONE-MARROW. × 600. 1-5. Various forms of bone-cells. 6. Eosinophilous cell. Techn. No. 59 *b*.

of the colored blood-corpuscles; these are the mother-cells of the colored blood-corpuscles, the hematoblasts (erythroblasts) (Fig. 76 and Fig. 77). Yellowish pigment-granules that appear in the different cells are regarded as the remains of disintegrated colored blood-corpuscles.

The *yellow marrow* consists of a connective-tissue reticulum con-

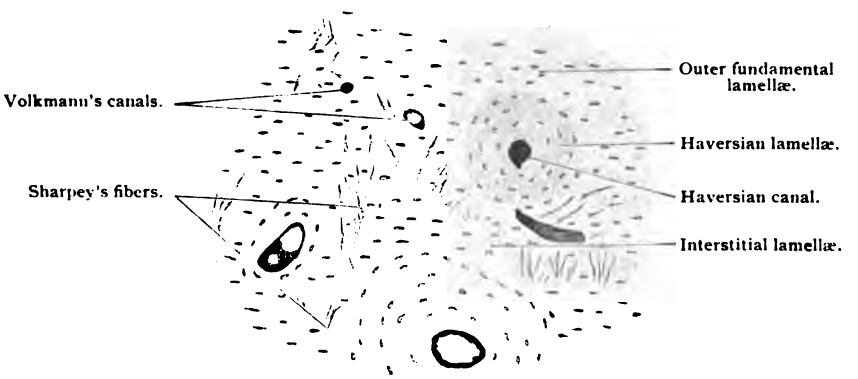


FIG. 78.—FROM A CROSS-SECTION OF THE FEMUR OF ADULT MAN. × 80. Techn. No. 57. The lamellæ can be recognized by the disposition of the lacunæ.

taining much fat. Marrow-cells and hematoblasts in yellow marrow are found only in the head of the humerus and the femur.

The *periosteum* is a compact connective-tissue membrane, in which two layers can be distinguished. The outer layer is characterized by its richness in blood-vessels and forms the connection with adjacent structures, tendons, fasciæ, etc.; the inner layer contains few blood-vessels,

but is very rich in elastic fibers running parallel with the long axis of the bone and spherical or spindle-shaped connective-tissue cells. Here and there on the inner surface a layer of cubical elements may be found, that are of importance in the development of the bone. The periosteum is sometimes firmly, sometimes loosely attached to the bone ; the attachment is secured by the blood-vessels passing to and from the bone and by Sharpey's fibers, which pierce the circumferential and adjacent interstitial lamellæ and extend in all directions (Fig. 78). In the tubular bones elastic elements of the inner layer of the periosteum penetrate the bone in company with Sharpey's fibers and without regard to the lamellar structure, run in the more superficial strata. Elastic fibers also occur that penetrate independently of Sharpey's fibers. In the bones of the vertex of the skull elastic elements are wanting.

The *blood-vessels* of the bone, the marrow, and the periosteum are in the closest connection with one another, and also with surrounding structures. Small branches (not capillaries) of the numerous arteries and veins of the periosteum enter the haversian and Volkmann's canals, which on the inner surface of the bone are in communication with the blood-vessels of the marrow. The latter is supplied by the nutrient artery, which on its way through the compact substance gives off branches to the same, and in the marrow breaks up into a rich vascular network. The veins that take up the capillaries of the marrow have no valves. *Lymph-vessels* with well-defined walls occur only in the most superficial layers of the periosteum.

The *nerves* are numerous and consist partly of medullated, partly of gray fibers. They enter the haversian canals, the bone-marrow, and the periosteum, and in the latter occasionally terminate in Pacinian corpuscles.

### The Articulations of Bones.

Two forms of articulations are recognized: 1, synarthroses, joints characterized by immobility ; 2, diarthroses, joints in which the bones are movable, one upon the other.

In *synarthroses* the bones are joined either by ligaments, the union constituting a *syndesmosis ;* or by the intervention of cartilage, forming a *synchondrosis.*

The ligaments are partly *fibrous bands*, possessing a structure like that of tendon, partly *elastic bands.* The latter are distinguished by the possession of numerous robust elastic fibers, which are never arranged in bundles or lamellæ, but are always separated by loose connective tissue. The ligamentum nuchæ, ligamenta subflava, and ligamentum stylohyoideum are elastic ligaments (Fig. 23, *C*).

The *sutures* also belong to the syndesmoses; they are short fibrous ligaments that extend from one serrated osseous edge to the other.

The cartilage in synchondroses is rarely only of the hyaline variety, but usually is in part fibro-cartilage (especially at the borders in contact with the bone) and in part hyaline, in which the cell-capsules are frequently calcified.

The intervertebral ligaments, which belong to the synchondroses, possess in their center a soft, gelatinous substance, the *nucleus pulposus*, that contains large groups of cartilage cells; it is the remains

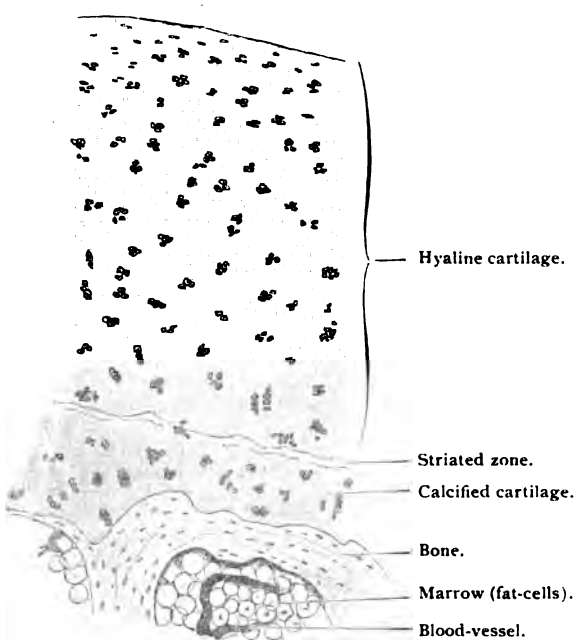


FIG. 79.—VERTICAL SECTION THROUGH THE HEAD OF A METACARPUS OF ADULT MAN. × 50. Techn. No. 60.

of the notochord, the embryonic precursor of the vertebral column. At the periphery of the intervertebral ligaments there is a narrow tendinous zone.

In *diarthroses* the parts entering into a joint are the articular ends of the bones, the capsular ligament, the marginal fibro-cartilages (*labra glenoidalia*), and the interarticular cartilages (*menisci*).

The articular ends of the bones are covered by a stratum of cartilage from 0.2 to 5 mm. thick thinning toward the edges. The superficial cartilage cells are flattened and placed parallel to the surface; those in

the median strata are rounded * and are often collected in groups; in the deepest strata the groups of cells are partly arranged in longitudinal rows, vertical to the surface of the bone; attached, but separated by a narrow striated belt, is a small zone of calcified cartilage interposed between and connecting the hyaline cartilage and the osseous tissue (Fig. 79). Not all the articular cartilages exhibit the structure just described; the cartilages of the costo-vertebral, the sterno-clavicular, the acromio-clavicular, and the maxillary articulations, and the head of the ulna are not hyaline, but fibro-cartilage; the distal articular surface of the radius is covered with dense fibrous tissue.

The *glenoid ligaments* and the *interarticular cartilages* do not exhibit the characteristic cartilage matrix; they consist of a compact fibrous connective tissue and of spherical cells. To the same category belong the so-called sesamoid cartilages. The tendinous sheath of the cuboid, however, contains genuine cartilage.

FIG. 80.—SYNOVIAL VILLI WITH BLOOD-VESSELS FROM A HUMAN KNEE-JOINT. × 50. The epithelium has fallen from the apex of the left villus, exposing the connective tissue. Techn. No. 61.

In the adult, nerves and blood-vessels are wanting in the articular cartilages, also in the interarticular cartilages and the glenoid ligaments.

The *capsular ligaments* consist of an external fibrous layer (*stratum fibrosum*) varying greatly in thickness, possessing a structure like that of the ligaments above described, and of an internal membrane, the *stratum synoviale*, the free inner surface of which is smooth and glossy; the outer layer of the latter is composed of loose elastic fibers and fibrillar connective tissue here and there containing fat-cells; within this is a thin lamella of parallel connective-tissue bundles, in which, toward the interior, there are small spherical or stellate cells, 11 to 17 $\mu$ in size, containing a large nucleus; the latter are sometimes few in number—at points subjected to more pressure—sometimes very abundant, and form an endothelial membrane three or four strata thick.

The synovial membrane (*stratum synoviale*) often forms folds containing fat that project into the synovial cavity and bears on its free surface

* Recently, the cells of the articular cartilages have been described as having processes which extend into the adjacent cartilaginous matrix. The cells of the deeper strata are said to possess lobulated nuclei.

the *synovial fringes* or *villi*, variously-shaped processes, mostly of microscopic size, which are particularly closely set on the edges of the joint-surfaces and bestow upon the synovial membrane a reddish, velvety appearance. They consist of connective tissue and are clothed by a single or double layer of endothelial cells.

The larger *blood-vessels* of the synovial membrane lie in the loose connective-tissue layer; from here the capillaries extend through the inner thin connective-tissue stratum and penetrate the villi. Some of the villi are nonvascular. The lymph-vessels lie close under the endothelium.

The *nerves* run in the loose connective tissue and in part terminate in Pacinian corpuscles.

The *synovia* contains more or less profoundly altered cells, fragments of cells, and oil-globules, all the product of physiologic processes of waste of the surfaces of the synovial membrane and articular cartilage; also albumin, mucus, and salts; the solids amount to six per cent., the remainder consists of water.

## The Cartilages.

The costal cartilages are of the hyaline variety; the matrix exhibits the peculiarities previously mentioned (p. 83), the cells frequently contain fat. The surface is enveloped by a compact fibrous membrane, the perichondrium, which consists of interlacing fibrous bundles and elastic fibers.

The articular cartilages are covered by the perichondrium only at their edges, not on their free surface. Where the cartilage and the perichondrium are in contact there is a gradual transition of the one tissue into the other and consequently the attachment between the two is very firm.

The perichondrium carries the nerves and the blood-vessels; the latter also run in excavated canals within growing cartilage. In the adult, cartilage is devoid of blood-vessels; the nutrition of the tissue depends upon diffusion from the surface. The costal cartilages in advanced life often contain blood-vessels because of beginning ossification.

The cartilages of the special-sense organs and of the respiratory organs will be described in the respective chapters.

## Development of Bone.

The bones are relatively late structures to appear. The development of the muscles, nerves, blood-vessels, brain, spinal cord, etc., is

already well advanced in the embryo at a time when not a trace of bone is present. At that period the skeleton is formed of hyaline cartilage. With the exception of certain parts of the cranium and nearly all the bones of the face, the entire skeleton is represented by cartilage. In the upper extremity, for example, the humerus, radius, ulna, carpus, and the skeletal parts of the hand consist of cartilaginous pieces, that are not hollow like the bones by which they are subsequently replaced, but solid throughout. The osseous skeleton then gradually appears in the place of the cartilaginous skeleton. All the osseous parts that in the embryo were preceded by cartilage are called *primary* or

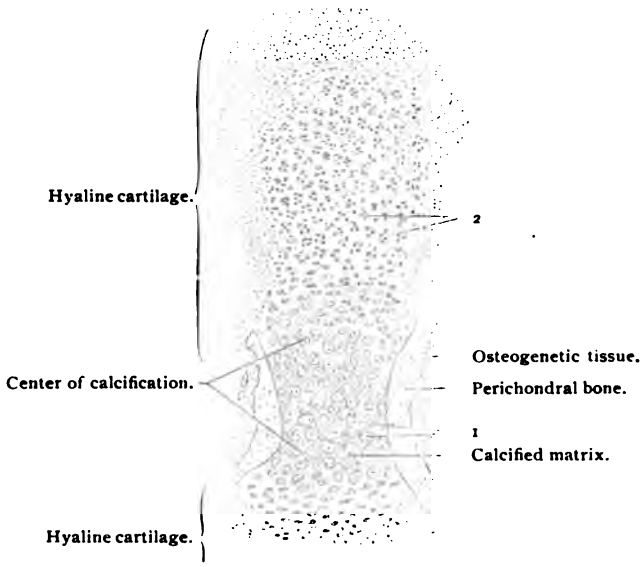


FIG. 81.—FROM A DORSO-PLANTAR LONGITUDINAL SECTION OF THE GREAT TOE OF A FOUR MONTHS' HUMAN EMBRYO. Two-thirds of the first phalanx represented. × 50. 1. Lacunæ enlarged and containing many cartilage-cells. The cells cannot be distinguished with this magnification, only their nuclei, which appear as minute dots. At 2, developing cartilage; cells in groups of three and four, each group produced by repeated division of one cartilage-cell. Techn. No. 62.

endochondral bone; the other bones, not preformed in cartilage, are named *secondary* or *intermembranous* bone.

The primary bones include all the bones of the trunk and extremities, the greater part of the base of the cranium (the occipital bone with the exception of the upper portion of the tabular part, the sphenoid bone with the exception of the internal pterygoid plate, the temporal bone with the exception of the squamous portion, the ossicles of the ear, the ethmoid bone, the inferior turbinal), and the hyoid bone.

The secondary bone includes the bones forming the sides and vertex of the cranium and nearly all the bones of the face.

## Development of Primary Bone.

Two modes of bone formation are here to be considered: 1, *endochondral formation*, formation of osseous tissue within the cartilage present, 2, *periosteal* (better *perichondral*) *formation*, formation of osseous tissue immediately surrounding, therefore upon, the cartilage. The phylogenetically older perichondral ossification usually begins earlier, but for didactic reasons will be described subsequently to the process of endochondral formation.

1. Endochondral Ossification.—The first indications of this process consist in changes at certain places within the cartilage; the cells

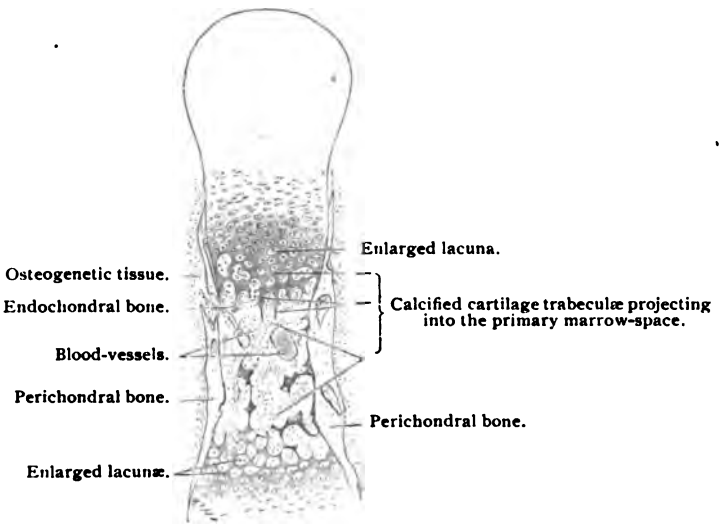


Fig. 82.—From a Dorso-palmar Longitudinal Section of the Finger of a Four Months' Human Embryo. Two-thirds of the second phalanx represented. × 50. The calcified trabeculæ are covered by a thin layer of endochondral bone. (More highly magnified in Fig. 83.) Techn. No. 62.

enlarge and divide, so that several lie in one lacuna; a deposition of lime salts takes place within the matrix, in consequence of which it becomes granular and dull, it calcifies. Such places may be recognized by the unaided eye, and are called *centers of ossification* (better, centers of calcification). The portions of the cartilage remote from the center of calcification continue to grow in thickness and length, while at the center growth ceases, and consequently the cartilage at this point appears constricted (Fig. 82). Meanwhile, on the surface of the center of calcification a tissue rich in blood-vessels and young cells, the *osteogenetic tissue*,*

* This is not a good name, inasmuch as the tissue has not originated from bone, but is to become bone.

has made its appearance. This penetrates into the cartilage and causes the breaking down of the calcified matrix; the cartilage-cells are set

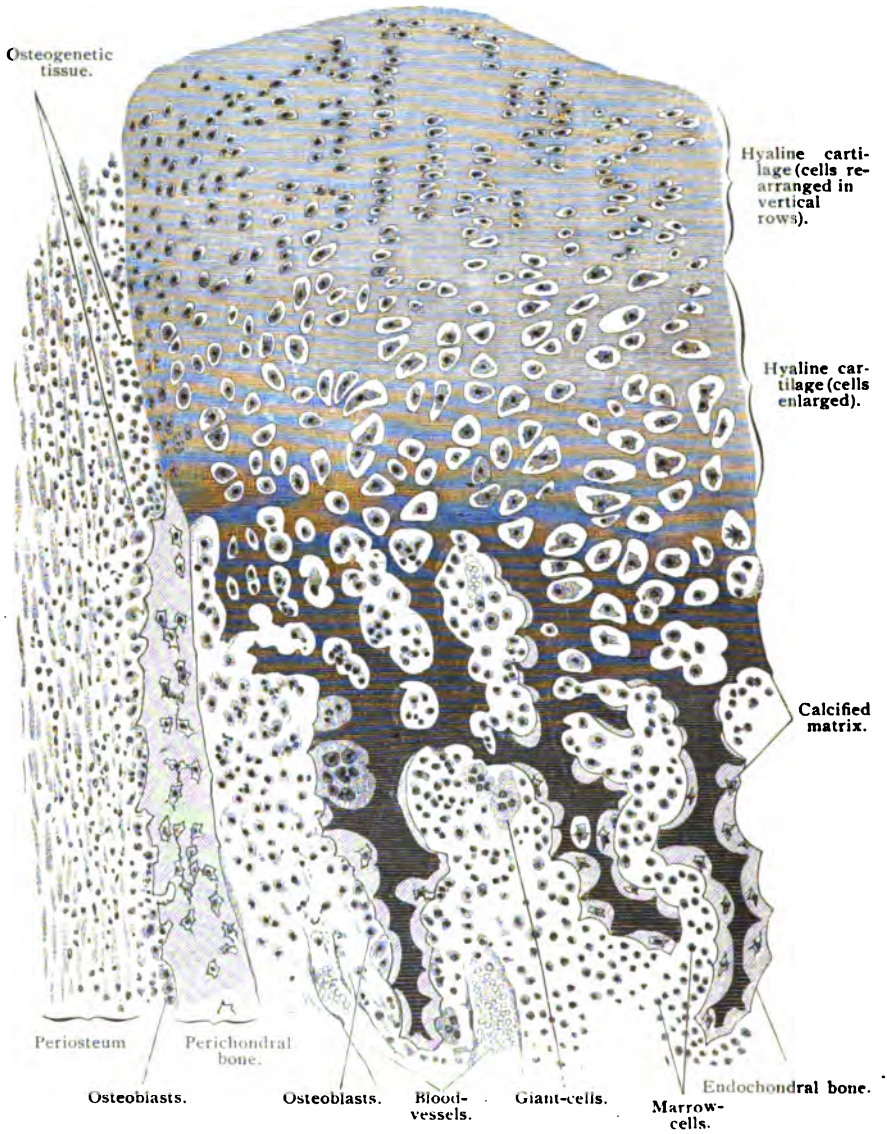


FIG. 83.—FROM A LONGITUDINAL SECTION OF THE PHALANX OF THE FIRST FINGER OF A FOUR MONTHS' HUMAN EMBRYO. × 220. In the endochondral bone irregular lacunæ with bone-corpuscles are seen. Techn. No. 62.

free and disintegrate. In this way a little excavation has arisen in the center of calcification; it is called the *primary marrow-cavity*.

These processes are repeated in the immediately surrounding carti-

lage ; that is, the matrix calcifies, the cartilage-cells enlarge, new portions of the cartilage break down, and as a result the primary marrow-space is gradually and continuously enlarged. At the same time the capsules of many cartilage-cells are opened, the cells degenerate, and the intervening calcified matrix projects into the marrow-space in the form of irregular processes or trabeculæ. The primary marrow-cavity now is a little bay

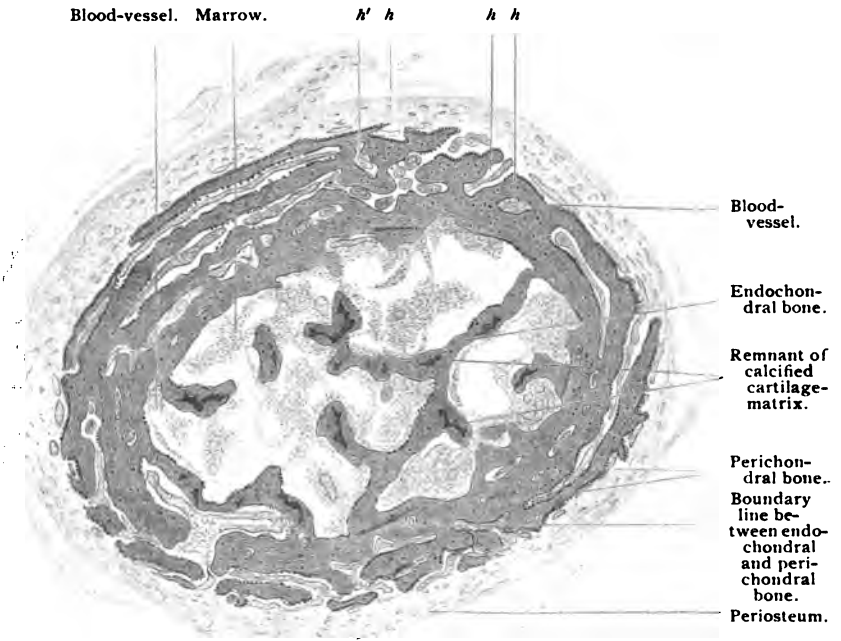


FIG. 84.—CROSS-SECTION OF THE UPPER HALF OF THE DIAPHYSIS OF THE HUMERUS OF A FOUR MONTHS' HUMAN EMBRYO. *h*. Developing haversian spaces; *h'*, blood-vessel. × 35. Techn. No. 62.

filled with blood-vessels and young cells. The fate of these cells in the further course of development varies greatly. They retain their original form and become marrow-cells, or they become fat-cells, or—and this is most important—they become bone-forming cells, *osteoblasts*. In the latter event, a number of cells arrange themselves in a single layer on the walls of the marrow-cavity and on the surface of the calcified trabeculæ and produce the matrix of osseous tissue.

As a result of the activity of the osteoblasts, the trabeculæ and the walls of the marrow-cavity are soon covered with a thin layer of bone-substance, gradually increasing in thickness. Thus step by step the former solid cartilage is transformed into spongy bone, the trabeculæ of which still contain a residue of calcified cartilage-matrix (Fig. 84).

2. PERICHONDRAL OSSIFICATION.—This mode of bone formation is also accomplished through the agency of the osteoblasts * derived from the osteogenetic tissue at the surface of the center of calcification (Fig. 81). Through the activity of the osteoblasts strata of plexiform osseous tissue are periodically formed on the surface of the cartilage; these osseous masses are distinguished from the endochondral bone by the absence of remnants of calcified cartilaginous matrix, because the perichondral bone is formed at the circumference and not in the interior of the cartilage. The formation of the first haversian canals may be observed in the perichondral bone (Fig. 84). The latter is not formed in a continuous layer of uniform thickness, but at frequent intervals depressions or recesses may be observed containing blood-vessels surrounded by osteoblasts (Fig. 84, *h h*); at first the recesses are open toward the periphery, but with the progressive development of the osseous strata they are closed in and then represent haversian canals. The osteoblasts enclosed within the canals produce new osseous strata, the future haversian lamellæ.

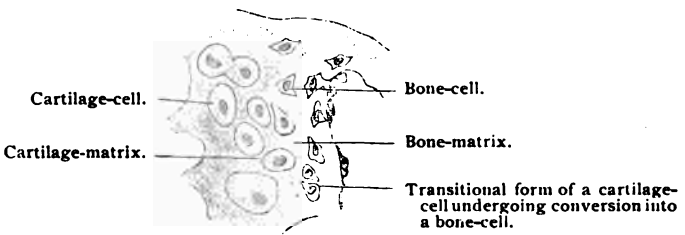


FIG. 85.—FROM A CROSS-SECTION OF THE LOWER JAW OF A NEWBORN DOG. × 240. Metaplastic type. Techii. No. 62.

By the absorption of the cartilage and by its substitution by osseous tissue, also by the deposition of bone-substance on its exterior, the piece of cartilage has become a bone.

The essence of the foregoing processes consists in an absorption of the parts of the primordial skeleton and in a reconstruction of the same by the development of bone-substance. This mode of bone formation is termed *neoplastic* in contradistinction to the rarer *metaplastic* mode, in which the cartilage is not destroyed but is ossified, and the cartilage-matrix becomes the bone-matrix, the cartilage-cells the bone-cells (as, for example, in the angle of the inferior maxilla) (Fig. 85).

* In the inner strata of the perichondral osseous cortex the osteoblasts are almost entirely absent; also in the region of the endochondral osseous trabeculæ the number of osteoblasts is smaller.

## Development of Secondary or Intermembranous Bone.

In this the foundation on which the formation of bone occurs is not cartilage, but connective tissue. Isolated bundles of connective tissue calcify; on these, osteoblasts derived from embryonal cells arrange themselves and produce bone in the manner above described (Fig. 86). The intermembranous bone is enclosed on all sides by connective tissue; when osseous tissue is in direct contact on one side with the cartilage, without the intervention of connective tissue, the resulting formation is not intermembranous, but perichondral bone.

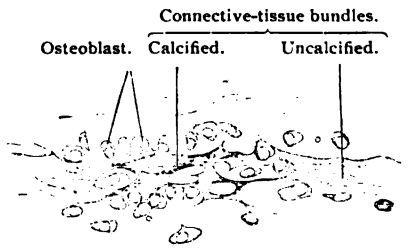


FIG. 86.—FROM A HORIZONTAL SECTION OF THE PARIETAL BONE OF A HUMAN EMBRYO. × 240. Techn. No. 62.

## Growth of Bone.

In *tubular bones* ossification in the diaphysis begins much earlier than in the epiphyses (in the humerus the center of ossification in the diaphysis appears in the eighth fetal week, in the epiphyses in the first year of life); blood-vessels grow into the calcified cartilage, which is transformed at first only by endochondral, later also by perichondral, formation into bone. The articular surfaces of the bone remain permanently cartilaginous; a narrow zone of cartilage between diaphysis and epiphysis, the *epiphyseal cartilage*, persists until the growth of the bone is completed. An active growth of cartilage is maintained here, that, by extension of the primary marrow-cavities of the diaphysis and the epiphyses, is continually being supplanted by bone. In this way the bone grows in length. Increase in thickness takes place by the constant "apposition" of new periosteal strata.

In the *short bones* ossification takes place, as in the epiphyses, at first by endochondral formation; after the absorption of the last superficial remnant of cartilage, a perichondral osseous shell is formed.

In the *flat bones* perichondral precedes endochondral formation.

*Intermembranous bones* grow in superficies and thickness by the formation of new osseous masses at their edges and on their surfaces respectively. As a consequence of the abundant deposition of bone-substance on the surface, the outer and inner tables of compact bone are formed, which enclose between them spongy bone; the latter in this

situation is termed diploë. The osseous masses at first possess a coarse fibered, later (from about the first year of life) a fine-fibered matrix.

## Resorption of Bone.

Immediately following the initial formation of osseous tissue, a contrary process, *resorption*, becomes perceptible, by which the calcified cartilage matrix and many parts of the primary and secondary bone are removed. Resorption occurs most actively in the tubular bones in the formation of the marrow-spaces, in a lesser degree in other bones, and on the surface of bones until their typical form is completed.*

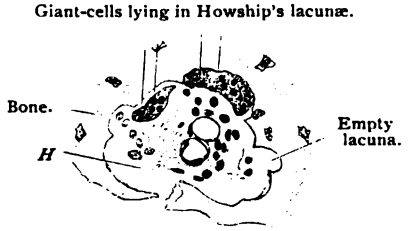


Fig. 87.—From a Cross-section of the Humerus of a Newborn Cat. × 240. *H.* Haversian space, containing two blood-vessels and marrow-cells. Techn. No. 62.

In the interior of the compact bone irregular excavations may be seen, the so-called haversian spaces, formed by the absorption of the innermost haversian lamellæ, which, however, may be partly filled again by the deposition of new osseous substance.

Wherever resorption of bone takes place, multinucleated giant-cells may be seen lying in pit-like depressions—*Howship's lacunæ*—which they have excavated in the bone. In this situation the giant-cells bear the name of *osteoclasts* (Fig. 87).

Even in the fully-developed skeleton the processes of apposition and resorption still occur at some places.

## TECHNIC.

No. 57.—*Ground Sections of Dried Bone.*—The bone must not be dried before maceration, but must be placed fresh for several months in water, which should be frequently changed. Then it is dried, and a piece held between two pieces of cork or cloth is clamped in a vice and with a compass-saw sections 1 or 2 mm. thick, transverse or longitudinal, are cut. Secure a section with sealing-wax to the under surface of a cork-stopper (the sealing-wax should also surround the section), dip the whole for a moment in water and then file it, first with a coarse, then with a fine file, until it is perfectly smooth ; the file must be frequently dipped in water, in order to wash off the adherent particles of bone and to prevent the heating of the sealing-wax by friction.

The section of bone should then be loosened by heating the sealing-

* For example, the femur of a three-year-old child contains scarcely any of the osseous tissue of the femur of the newborn child.

wax and the smooth side stuck fast to the stopper. It must now be filed until it is so thin that the sealing-wax can be seen through it. The whole should then be placed in 90 per cent. alcohol, in which within a few minutes the section becomes loosened from the cork. Now moisten a coarse whetstone with water, rub it with a second whetstone until the surface is covered with a little grinding-paste ; lay the section in it, place a smooth cork upon it (one without cracks), and with a circular motion grind it on both sides ; it is not necessary to glue the section to the cork. The section when sufficiently thin is transparent ; this is to be ascertained by drying it between pieces of filter-paper and examining with the low power. It should then be ground on a fine whetstone, in the same manner as on the coarse, and when both sides are smooth it should be dried with filter-paper and polished. To do the latter, nail a piece of wash-leather smoothly on a board, sprinkle it with chalk, and with the tip of the finger rub the section to and fro on it. In this way the previously dull section acquires shining surfaces. The adherent powder may be removed by rubbing the section on fresh wash-leather. The finished section is to be placed dry on a slide and the cover-glass secured by means of cement (p. 45).

Examine first with the low, then with the high power (Fig. 34). If the section is thick, it may be impossible to examine it with the high power, since then the objective cannot be brought near enough to the preparation. The bone lacunæ and bone canaliculi are filled with air and with the customary illumination of the object from below appear black.

No. 57 *a*.—*Sharpey's Fibers*.—Prepare a cross-section of the middle of the shaft of a tubular bone, preferably of a young individual, according to the method given in No. 56. Place the finished dry section for from two to five minutes in 4 c.c. of turpentine and then mount in damar. The fibers, invisible in the sections produced by other methods (No. 56 and 58), can be plainly seen, even with the low power (Fig. 78).

No. 58.—*Haversian Canals and Lamellæ*.—Select the metacarpal bone of an adult ; after four weeks' fixation in Müller's fluid, and hardening in alcohol, decalcify in nitric acid (p. 34), harden again, and cut transverse and longitudinal sections. The compact structure of larger bones (the femur, for example) requires too much time (several weeks) for decalcification. The periosteum should be allowed to remain on the bone. For longitudinal views of haversian canals very thick sections (0.5 mm. or more) must be cut. Mount in dilute glycerol (Fig. 74). Neither are very thin sections necessary for transverse views and lamellar systems ; the lamellæ are best seen if the section be examined in a drop of distilled water and the mirror turned so that the object is only half illuminated ; thus, too, the striæ produced by the bone canaliculi, running vertically to the lamellæ, are best seen (Fig. 75). Mount in dilute glycerol, which, however, renders the lamellar systems partially indistinct. Not every part of the bone exhibits all the lamellar systems ; the outer and also the inner ground lamellæ are frequently wanting. In sections taken near the epiphyses the transition of the compact substance into the

trabeculæ of the spongy bone may be seen. The bone lacunæ and bone canaliculi are much less distinct in moist preparations than in dried ground sections, because the contained air has been displaced by the mounting medium. (Compare Fig. 34 with Fig. 35.)

Not infrequently the concentric lamellæ of the haversian systems are found to be interrupted by an irregular line. Up to this line the osseous tissue previously formed has been again resorbed. All that which lies within the line is newly-deposited bone-substance. These formations are, therefore, partially filled Haversian spaces (Fig. 75, *h*).

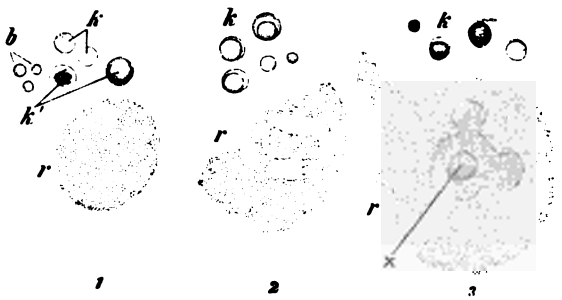


FIG. 88.—ISOLATED ELEMENTS OF FRESH BONE-MARROW FROM THE VERTEBRA OF A CALF. × 560. 1. In salt solution. 2. Stained with picrocarmine. 3. After treatment with acidulated glycerol. *k*, Marrow-cells; *k'*, two marrow-cells containing masses of pigment-granules, the cell on the right seen from the side, the cell on the left from the surface; *b*, nonnucleated colored blood-corpuscles; *r*, giant-cells; in the one on the right the nucleus is dividing by constriction, and two of the future new nuclei are seen from the side, another, x, from the surface.

No. 59.—*Red Bone-marrow.*—*a.* Compress the vertebra (cut in half) or the rib of a calf in a vice or with tongs; with a pipet take up a small drop of the liquid thus expressed, transfer it to a slide and, without the addition of any other fluid, apply a small cover-glass or, better, a fragment of a cover-glass. Examined with the high power red blood-corpuscles, hematoblasts, marrow-cells of different sizes, and giant-cells will be seen, but not always their nuclei (Fig. 88, 1). Add a drop of picrocarmine (p. 48); the nuclei become red in from one to two minutes, but are still pale (Fig. 88, 2). If the picrocarmine is displaced by salt solution and then by dilute acidulated glycerol, the nuclei acquire a deep color and sharp contours (Fig. 88, 3). Occasionally giant-cells are sought in vain. Human ribs are often usable.

*b.* To make permanent preparations, proceed as follows: With a thin cover-glass take up a drop of the marrow expressed from a rib and make two cover-glass preparations as directed in No. 39. Since the marrow does not diffuse as readily as the blood between the two cover-glasses, make slight pressure upon them before slipping them apart. They should not be allowed to dry, but should be placed at once in a concentrated aqueous solution of sublimate solution (5 gm. in 100 c.c. of distilled water). At the end of ten minutes transfer the cover-glasses to 20 c.c. of distilled water, which is to be changed in about five minutes. In ten minutes place them in 5 c.c. of diluted eosin (p. 37, 3 *b*) for from one to

five minutes, then wash for a moment in distilled water and transfer them to 5 c.c. of filtered Hansen's hematoxylin; after one or two minutes place them for five minutes in distilled water; remove the water by means of filter-paper placed at the edge of the cover-glass and place them in 95 per cent. alcohol (not longer than one minute, lest the eosin be extracted), then in pure oil of bergamot for three minutes. With a cloth carefully remove the oil from the film-free surface of the cover-glass, place a drop of damar on the surface containing the film of marrow, and invert the cover-glass on a slide. The colored blood-corpuscles and the protoplasm of the hematoblasts are stained a brilliant red, the protoplasm of the remaining cells gray-violet; all the nuclei are blue. Cells containing oxyphile (eosinophile) granules are often found (Fig. 76). Cells with neutrophile and basophile granules are obtained by treating bone-marrow according to Techn. No. 42.

No. 60.—*Articular Cartilage.*—Select the head of the metacarpal bone of an adult, and treat it according to the method given in No. 57. Cut longitudinal sections and mount them in dilute glycerol (Fig. 79). The parallel streaks often present in the hyaline cartilage are produced by the razor. The granules of the calcified cartilage have disappeared in consequence of the process of decalcification to which the tissue was subjected.

No. 61.—*Synovial Villi.*—From a cadaver, as fresh as possible, cut out a piece about 4 cm. long of the capsular ligament at the edge of the patella, and with the scissors cut a strip 2 or 3 mm. broad from the reddish, glossy, velvety inner surface of the same, moisten it with a drop of salt solution, and without a cover-glass examine it with the low power. At the edges of the tissue the villi may be seen; their blood-vessels often still contain blood-corpuscles. The refractive nuclei of the endothelial cells lie close beside one another (Fig. 80).

If it is desired, the preparation may be stained under the cover-glass with picrocarmine and mounted in diluted glycerol (p. 48), but much of the original beauty is lost.

No. 62.—*Development of Bone.*—Human embryos four or five months old, embryos of the sheep, pig, or cow, from 10 to 14 cm. long (measured from the tip of the snout to the root of the tail), are suitable. The latter are readily obtained at the slaughter-house; the entire uterus should be ordered. Place the embryos in toto (2 or 3 in 1 liter) in Zenker's fluid for forty-eight hours. Then wash in running water for forty-eight hours, and harden in 200 to 400 c.c. of gradually-strengthened alcohol (p. 33). After the embryos have lain one week or longer in 90 per cent. alcohol, to which tincture of iodin has been added (p. 32), cut off the head, the extremities close to the rump, and decalcify them in 200 c.c. of distilled water to which 2 or 4 c.c. of pure nitric acid have been added. In two or five days, during which the decalcification medium must be changed about three times, the extremities are to be taken out (the head is probably not yet decalcified, and must remain in two per cent. nitric

acid for several days longer) and washed from one to six hours in running water, and again hardened in gradually-strengthened alcohol. After they have lain five days in 90 per cent. alcohol, cut the extremities into pieces 1 cm. long, which, should they still be too soft, may be placed for one or two days in 30 c.c. of absolute alcohol.

The vertebræ and the ribs also furnish instructive specimens.

To obtain sections showing the *first processes* in the development of bone, embed in liver the phalanges and metacarpal bones (the latter are very long in the animals mentioned), and make longitudinal (sagittal) sections, from the flexor to the extensor surface; to be good the sections must be taken in the axis of the extremities; those taken from the margin exhibit pictures that are unintelligible.

For *more advanced stages* make chiefly transverse sections of the humerus and femur. Sections through the diaphysis show more perichondral, sections through the epiphyses more endochondral bone.

The most beautiful examples of *osteoblasts* are obtained in cross-sections of the inferior maxilla; they are also valuable as preparations showing the development of teeth.

For still *later stages* the skeleton of newborn animals is useful; their phalanges show tolerably early stages in the process, their carpal bones the first stages. The decalcification requires somewhat more time (up to eight days).

For *intermembranous bone* select the parietal and frontal bones of embryos; make horizontal sections.

The sections are to be stained in 4 c.c. of Hansen's hematoxylin for from two to ten minutes, transferred to 10 c.c. of distilled water for ten minutes, then to 4 c.c. of picrocarmine for ten minutes (p. 38), to 20 c.c. of distilled water for from fifteen minutes to one hour, and mounted in damar (p. 45).

If the staining is successful, the cartilage (especially the calcified portions) is blue, the bone red. Occasionally the cartilage does not stain well; then place the sections in 5 c.c. of distilled water plus 5 drops of filtered hematoxylin solution. In from six to fourteen hours the cartilage will become blue. The picrocarmine staining of bone often is not uniform; the youngest portions of the bone, the margins of the osseous trabeculæ, for example, are often the more brilliantly stained.

# IV. THE ORGANS OF THE MUSCULAR SYSTEM.

The muscular system is composed of a large number of contractile organs, the muscles, which consist of cross-striated muscle-tissue and are joined to the skeleton, the skin, the viscera, etc., by the intervention of special connective-tissue formations, the *tendons*, and by accessory apparatus of similar structure, the *fasciæ*, *tendon-sheaths*, and *bursæ*.

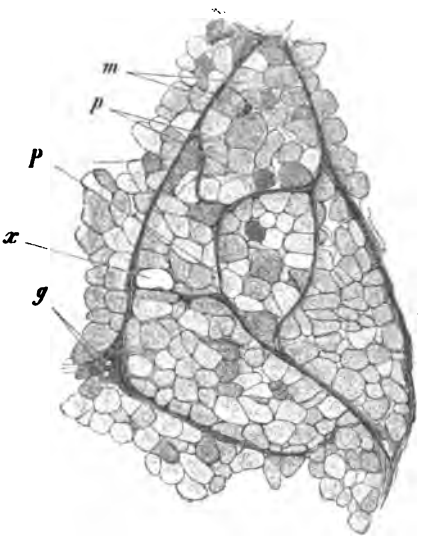


FIG. 89.—FROM A CROSS-SECTION OF THE ADDUCTOR MUSCLE OF A RABBIT. *P*. Perimysium, containing two blood-vessels, at *g*; *m*, muscle-fibers; many are shrunken and between these the endomysium, *p*, can be seen; at *x* the section of muscle-fiber has fallen out. × 60. Techn. No. 63.

Each *muscle* is composed of striated muscle-fibers, which as a rule are longitudinally disposed, so that they lie side by side and behind one another, and are held together by loose connective tissue, the *perimysium*. Interlacing is rare, but occurs, for example, in the tongue. Neighboring muscle-fibers never are in direct contact, but each individual fiber is enveloped in a delicate connective-tissue sheath, the perimysium of the single muscle-fiber, or *endomysium*, which is joined to neighboring sheaths (Fig. 89, *p*). A number of muscle-bundles * form a muscle, the surface of which is covered by a still thicker connective-tissue membrane, the perimysium externum, or *epimysium*. The several sheaths are connected with one another.

The perimysium is composed of fibrillar connective tissue and numerous fine elastic fibers,† occasionally contains fat-cells, and conveys

* The grouping of the primary bundles in secondary bundles, that in a certain number of instances are grouped in tertiary bundles, that finally unite to form a muscle, is an arbitrary division, and in many preparations cannot be recognized.

† In the epimysium they are present in great abundance.

the nerves, blood-vessels, and lymph-vessels. The endomysium contains only capillaries and terminal branches of nerves.

The post-embryonal increase in the thickness of the muscles depends less on the multiplication than on the growth in thickness of the already existing muscle-fibers.

The *tendons* are characterized by the parallel course of their fibers, by their firm union, and by the scarcity of elastic fibers. They are composed of dense, fibrous connective-tissue bundles, the "tendon-bundles," which are held together by looser connective tissue and form the so-called secondary bundles. Each secondary bundle consists of a number of parallel fibrillæ running a perfectly straight course and united by a small

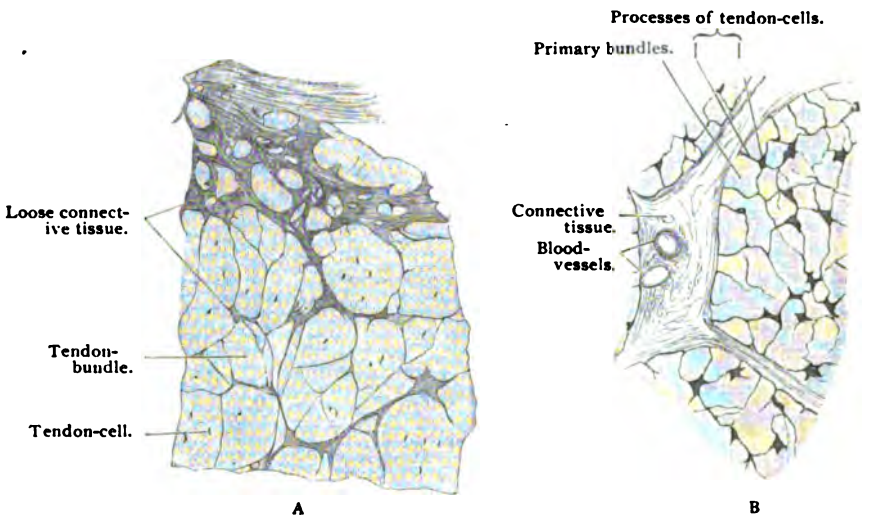


FIG. 90.—A. FROM A CROSS-SECTION OF DRIED TENDON OF ADULT MAN. × 50. Techn. No. 64. B. From a cross-section of tendon fixed with chromic acid of adult man. Techn. No. 65.

amount of cement-substance in so-called primary bundles. Between the primary bundles lie the cellular elements of the tendon, fusiform, stellate, quadrate, or flat cells, arranged in longitudinal rows, which, curved like concave tiles, partially clasp the primary bundles and unite with one another by means of processes. Elastic fibers are found chiefly in the loose connective tissue; in the dense tendon-bundles they are very scarce and occur in the form of a fine, wide-meshed network.

The union of the muscles with tendons and fibrous membranes (periosteum, fascia) is effected by an extension of the endomysium of the muscle-fiber into these structures and the blending of the tissues; the sarcolemma takes no part in this but, closely investing the muscle-fiber, terminates as a closed sheath with pointed or obliquely blunted ends.

The radiating cross-striped muscle-fibers in the skin attach themselves to the connective tissue of the corium by pointed or forked ends.

The *fasciæ* in part exhibit the same structure as the tendons and in part they are fibrous membranes richly provided with elastic fibers. The latter is the case when they form sheaths for the muscles and do not furnish surfaces for the attachment of the muscle-fibers.

The *tendon-sheaths* and the *bursæ* consist of a layer of connective tissue and elastic fibers, varying in thickness, the inner surface of which is covered patchwise by a simple stratum of polygonal, connective-tissue endothelial-cells. Where the endothelium is wanting the connective tissue is dense and rich in rounded elements resembling cartilage-cells.

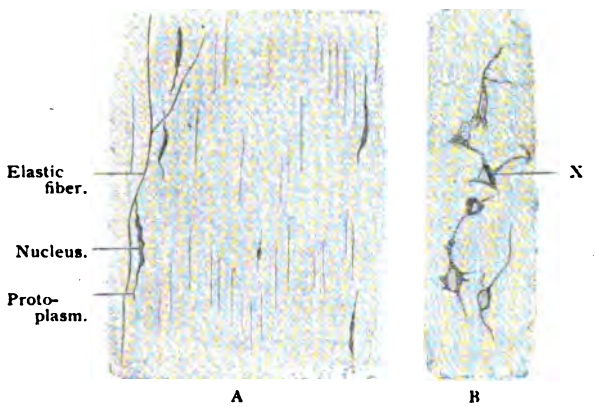


FIG. 91.—TENDONS FROM RAT'S TAIL. × 240. A. Tendon-cell viewed in profile. B. From the surface. At X the nucleus is bent so that it is seen partly in profile (the shaded portion) and partly from the surface (the light portion). Techn. No. 65.

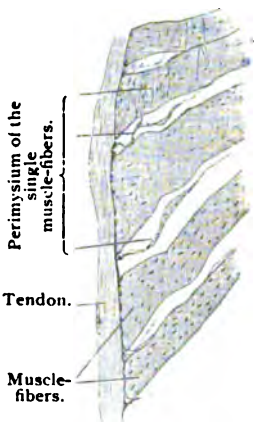


FIG. 92.—FROM A SAGITTAL LONGITUDINAL SECTION OF THE GASTROCNEMIUS OF THE FROG. × 50. The uppermost transverse line represents the perimysium seen from the surface. Techn. No. 67.

The majority of the tendon-sheaths have small vascular processes exactly like the synovial fringes.

The *blood-vessels* of striated muscles are very numerous and evenly distributed; the capillaries are among the thinnest in the human body and form networks characterized by elongated rectangular meshes, closely surrounding the individual fibers. The veins are provided with valves even in their smallest branches. The *lymph-vessels* are few in number and follow the ramifications of the smaller blood-vessels.

For the nerves, partly sensory and partly motor, of cross-striped muscle see the Peripheral Nerve-endings.

The *blood-vessels* of the tendons and the thinner fasciæ are very scarce, and are contained only in the loose connective tissue surrounding

the tendon-bundles; the tendon-sheaths and the bursæ have a rich vascular supply. *Lymph-vessels* are found only on the surface of the tendons.

The medullated *nerves* of tendons terminate in part in a close plexus of gray nerve-fibers and in part pass into spindle-shaped expansions of the tendon, the so-called *tendon-spindles*, where they end in structures resembling, but more richly branched than, the motorial end-plates. End-bulbs and Pacinian corpuscles are found in tendons, fasciæ, and tendon-sheaths.

## TECHNIC.

No. 63.—*Bundles of Striped Muscle.*—Select a muscle in which the fibers have a parallel disposition (for example, the adductor of the rabbit) and with a sharp razor make a deep incision transverse to the course of the fibers and 2 or 3 cm. below a second incision; connect these by longitudinal incisions and, without traction, carefully remove the piece thus mapped out. For fixation place it in 100 c.c. of 0.1 per cent. chromic acid (p. 31). After two weeks wash it in running water and harden in 50 c.c. of gradually-strengthened alcohol (p. 33). Cut cross-sections and examine them unstained in diluted glycerol (Fig. 89). The muscle-fibers vary greatly in thickness; the smallest are sections through the ends of the fibers. Although the muscle-fibers are cylindrical and should therefore in section appear circular, they have an irregularly polygonal outline due to mutual pressure. The color of the sections is very different, some are quite dark, others quite clear. The cause of this phenomenon is unknown to me. The endomysium is best seen with the high power (240 diameters).

No. 64.—*Tendons.*—Cut from a tendon a piece 5 or 10 cm. long, and let it dry in the air (but not in the sun). Thin tendons (*e. g.*, that of the flexor digitorum pedis) at room-temperature are sufficiently dry in twenty-four hours. Thicker tendons require several days. With the scalpel (not the razor) cut a smooth transverse surface and then cut thin shavings from the tendon, supporting it on the thumb of the right hand and with the remaining fingers grasping the scalpel (the manipulation is the same as in sharpening a pencil). Throw the shavings into a capsule containing distilled water and in two minutes examine in a drop of the same medium (Fig. 90, A). To preserve, stain in 3 c.c. of picrocarmine for five minutes and mount in dilute glycerol. Very frequently a streak may be seen extending across the entire section; this is produced by the knife.

Place another section, unstained, in a drop of water on a slide; treat it under the cover-glass with a drop of acetic acid; the edge of the section soon exhibits swollen convoluted bands (acetic-acid reaction of connective tissue).

No. 65.—For the study of the *minute structure of tendon, its cells* and *their processes*, place a thin tendon, as fresh as possible (that of the

palmaris longus muscle), in pieces 3 cm. long in 100 c.c. of 0.5 per cent. chromic acid for at least four weeks. The chromic acid should be changed several times during this period. Then wash the tissue in running water one or two hours and harden it in about 40 c.c. of gradually-strengthened alcohol (p. 33). The sections should be cut with a very sharp razor; often the tendon is so brittle that it falls to pieces in cutting. The sections need not be very thin. Mount them unstained in diluted glycerol. Examined with the low power and reflected light (with the mirror muffled) they yield beautiful pictures, better than the preparations made like Techn. No. 63. With the high power they resemble Fig. 90, B. The black zigzag spaces are partly occupied by tendon-cells.

No. 66.—*Tendon-cells.*—From the tail of a rat or mouse cut pieces of tendon from 0.5 to 1 cm. long and place them in 5 c.c. of alum-carmine. The following day (or later) transfer the swollen pieces to a dry slide and rapidly tease them (p. 28). It is not necessary to separate the tendon into very small bundles, but care should be taken that the bundles lie straight. Then cover the preparation with a drop of distilled water and a cover-glass. With the low power the rows of cells appear for the most part as dark streaks; they are the cell-nuclei seen in profile. In surface views the nuclei appear dull red. The body of the cells, the protoplasm, can only be seen with the high power; viewed laterally, it appears as a sharp, dark streak (Fig. 91, A); from the surface, paler and delicate (Fig. 91, B). Not infrequently the cells are folded, so that they are visible partly from the edge and partly from the surface. The connective-tissue fibers may be occasionally distinguished as delicate parallel lines; the fine elastic fibers with their sharp contours are always distinct. The focus should be changed by means of the micrometer-screw, and the different planes of the section examined. If the cells are not distinct add a drop of acetic acid (p. 48). To preserve, displace the water with diluted glycerol.

No. 67.—*Muscle and Tendon.*—Remove the skin from the hind leg of a frog just killed, and with scissors cut off the leg above the knee-joint, just above the origin of the gastrocnemius. Fix it in 50 c.c. of Kleinenberg's picrosulphuric acid (p. 21). After twenty-four hours transfer it directly to 50 c.c. of 70 per cent. alcohol for gradual hardening. In about six days cut off the muscle with a piece of the tendo-Achillis, and stain it in bulk in borax-carmine (p. 37). Then harden again in 90 per cent. alcohol. Cut sagittal longitudinal sections, placing the razor first on the tendon occurring on the posterior surface of the muscle. Mount in damar (p. 45). Very often not a trace of the cross-striation of the muscle-fibers is to be seen (Fig. 92).

# V. THE ORGANS OF THE NERVOUS SYSTEM.

## I. THE CENTRAL NERVOUS SYSTEM.*

### THE SPINAL CORD.

*Topography.*—The spinal cord consists of a white and a gray substance, that are distinguishable by the unaided eye. The arrangement and the relation of these substances are best recognized in cross-sections of the spinal cord.

The *white substance* encircles the gray substance, and is partially divided by a deep anterior cleft, the *anterior median fissure*, and a posterior *septum* (formerly called the posterior median fissure) into a right and a left half. Each half is subdivided by the furrows marking the exit of the anterior and the posterior roots of the spinal nerves into a large *lateral column*, an *anterior column*, and a *posterior column*. In the lower cervical and the upper thoracic regions two divisions may be distinguished in the posterior column, of which the median portion is named the *column of Goll* (funiculus gracilis) and the lateral portion the *column of Burdach* (funiculus cuneatus). The anterior columns are united by the *white commissure* at the bottom of the anterior median fissure.

The *gray substance* in cross-section appears in the form of an H and in its entirety consists of two lateral columns, which are connected by a horizontal lamella, the *gray commissure*. On each column thick *anterior cornua* and slender *posterior cornua* may be distinguished. Adjoining the lateral portions of the anterior horns, in the same frontal plane with the central canal, are the *lateral cornua*, which are especially well developed in the upper thoracic region. From the front boundary of the anterior cornua the *anterior roots* of the *spinal nerves* emerge in several bundles, while the *posterior roots* enter at the postero-median side

* I shall confine myself here to a brief account of the topography and histology of the spinal cord and the brain. An exhaustive presentation of the architecture of the central nervous system, the paths of the nerve-fibers, and the complicated origins of the cranial nerves in the "nuclei" of the oblongata would exceed the limits of this "Histology." The student is referred to special text-books, of which Edinger's "Vorlesungen über den Bau der nervösen Centralorgane" is recommended.

of the posterior cornua. Laterally, at the base of the posterior cornua, a net-like mass of gray substance, the *reticular process* (formatio reticularis), is found ; at the median side of the posterior horn, near the gray commissure, lies the *column of Clarke* (dorsal nucleus), well defined in the whole length of the thoracic and in the upper part of the lumbar region of the cord. At the summit of the posterior horns a glistening, jelly-like mass, macroscopically easily perceptible, the *substantia gelatinosa* (*Rolando*), may be distinguished. Posteriorly to this is the small *zona spongiosa*, at the dorsal edge of which is found the border zone, *zona*

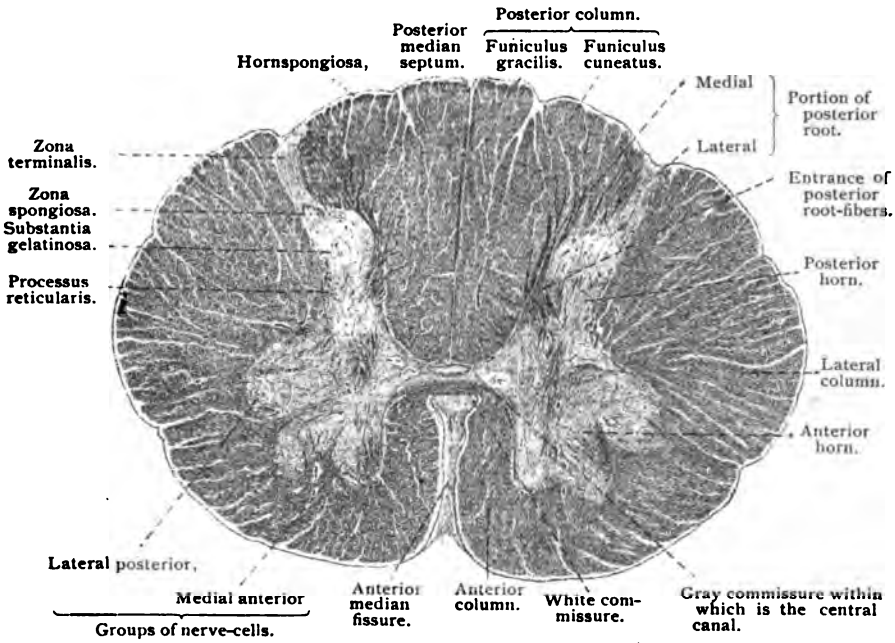


FIG. 93.—CROSS-SECTION OF THE CERVICAL ENLARGEMENT OF THE HUMAN SPINAL CORD. × 7. Techn. No. 67.

*terminalis*, an area of cross-sectioned thin nerve-fibers. In the gray commissure lies the cross-section of the *central canal*, which extends through the whole length of the spinal cord and is surrounded by the *substantia grisea centralis*. The *central canal* is from 0.5 to 1 mm. in diameter ; not infrequently it is obliterated. The portions of the gray commissure in front of and behind the central canal are respectively named *anterior* and *posterior gray commissure*. In man the latter is the smaller. From the entire periphery of the gray substance coarser or finer processes, the *septula medullaria*, radiate into the white substance. In the cervical and lumbar enlargements of the spinal cord the gray matter is

more powerfully developed than in the thoracic region; there is a corresponding variation in the form of the H. The end of the *conus medullaris* consists almost wholly of gray substance.

*Minute Structure.*—The *gray substance* will be first considered, a knowledge of its composition being essential to the comprehension of the structure of the white substance. The gray substance consists of multipolar nerve (ganglion)-cells, that with their dendrites and nerve-processes form a dense nervous tangle, the "nerve-felt" (*neuropilem*). This felt is penetrated by nerve-fibers, partly proceeding from the white columns, partly from the posterior roots; the whole is supported by a framework of *neuroglia.*

We have to consider first the nerve-cells, then the nerve-fibers; the neuroglia, which also occurs in the white substance, will be described at the conclusion of the entire recital.

The *nerve-cells*, in accordance with the relations and distribution of their nerve-process, are divided into (1) motor cells, (2) column-cells, and (3) internal cells.*

The *motor nerve-cells* (*rhizoneurons*) lie in two groups † in the anterior horn. They possess a large cell-body (67 to 135 $\mu$) and long dendrites, extending far into the surrounding substance; the nerve-process emerges from the summit of the anterior cornu, makes an oblique descent through the white substance, at the same time receives a medullary sheath and becomes the axis-cylinder of a medullated nerve-fiber. Occasionally the axis-cylinder process gives off a few insignificant lateral

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* *Editor's remark:* A classification and nomenclature based upon the behavior and distribution of the axis-cylinder have recently been suggested in America, that, in many respects, appear to me to be appropriate and natural, and have been widely accepted. According to this two chief groups are distinguished, namely: I, *axoneurons*, and, II, *ganglioneurons.*

I. The **axoneurons** embrace all those neurons the cell-body (nerve-cell) of which lies *in the interior of the spinal cord or the brain.* Corresponding to the different behavior of the nerve-process, they are further divided into two subordinate groups, namely:

(*a*) *Rhizoneurons*, the nerve-process of which leaves the spinal cord through the *anterior root* (they comprise the motor nerve-cells), and—

(*b*) *Endaxoneurons*, the nerve-process of which does *not* leave the spinal cord. Among these we may distinguish (1) those the nerve-process of which enters the different columns of the white substance (*column-cells*), and (2) those the nerve-process of which within the gray substance rapidly breaks up into its terminal ramifications (*internal cells*).

II. The **ganglioneurons** represent those neurons the cell-body of which lies within the *spinal ganglia or the cerebral ganglia* and that stand in connection with the central nervous system only by means of their central process.

† An antero-median and postero-lateral group, separate in the cervical and lumbar enlargements, but in the uppermost cervical and in the thoracic regions united in a single cluster (Fig. 93). In longitudinal sections it may be seen (conspicuously in amphibians) that the cell groups, governed by the origin of the single roots, have a correspondingly typical segmental arrangement.

twigs (collaterals) before leaving the gray matter. It leaves as a constituent part of an anterior (ventral) root-fiber bundle of the spinal cord. All anterior root-fibers arise from the motor cells of the anterior horn, from those of the same, not the opposite, side.

The *column-cells* (*Strangzellen*, *endaxoneurons*) constitute the chief mass of the nerve-cells of the gray substance, and in this lie everywhere (except in the places occupied by the motor nerve-cells), partly scattered, partly in groups in the lateral horn and in the dorsal nucleus. The majority are smaller than the motor nerve-cells and possess few, little-branched, but far-reaching dendrites. Their nerve-process, after sending

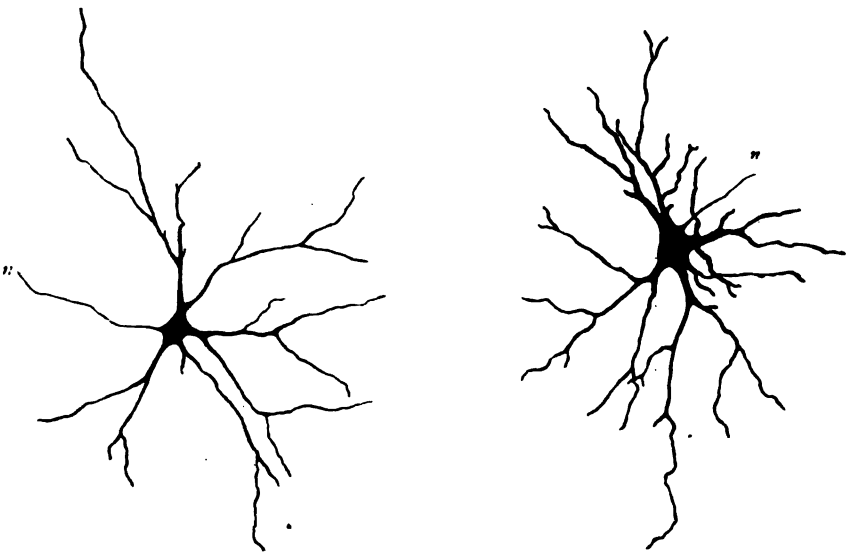



FIG. 94.—TWO FORMS OF MOTOR NERVE-CELLS FROM THE ANTERIOR HORN OF THE SPINAL CORD OF A RABBIT. *n*. Nerve-process. × 60. Techn. No. 70. (Schaper.)

off numerous collaterals in the gray substance, enters the white substance —in the anterior or lateral column, very rarely the posterior column— either on the same or on the opposite side. Cells of the latter kind are sometimes termed *commissure-cells*,* because the nerve-process passes through the anterior gray commissure before entering the white substance. Having arrived in the white substance, the nerve-process of the majority

* The commissure-cells occupy an area which, arch-like, embraces the central canal on the ventral side; there they are of conspicuous size, approaching that of the motor cells of the anterior horns. Also farther back, in the median division of the gray substance, scattered commissure-cells occur, but they are wanting in the posterior horns.

of the column-cells * divides into a vertical ascending and descending "stem-fiber," that in its course parallel to the longitudinal axis of the spinal cord sends off lateral twigs (collateral fibers), which return to the gray substance, where they terminate in tufts of free fibrils; the stem-fibers themselves finally terminate like the collateral fibers. The collateral fibers that enter from the anterior columns penetrate the anterior cornua singly or in bundles, where they weave a net around the large motor cells; they are especially numerous in the antero-lateral curve of the anterior horn; not less numerous are the collateral fibers coming from the lateral columns. The spindle-shaped "marginal cells" lying in the zona spongiosa also belong to the column-cells. In the adult, all

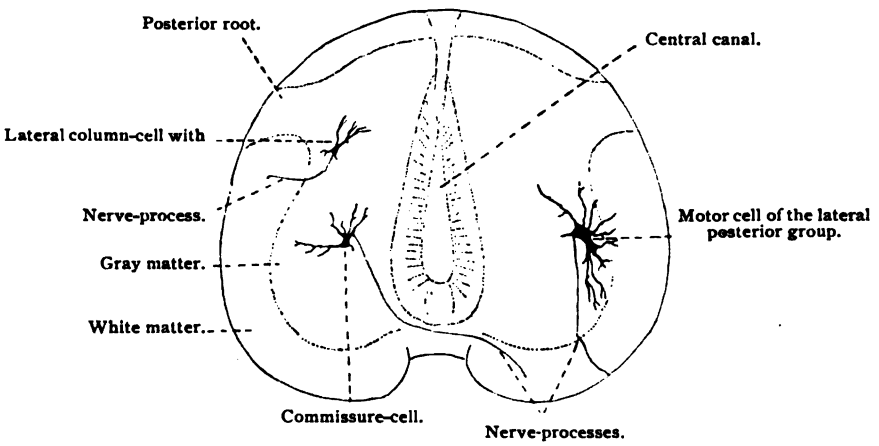



FIG. 95.—CROSS-SECTION OF THE SPINAL CORD OF A SEVEN-DAY-OLD EMBRYO CHICK. × 80. The white matter is but slightly developed, the central canal is still very large. Techn. No. 70.

the nerve-processes of the column-cells are enveloped in a medullary sheath.

The cells so far described are characterized by their long nerve-process; they belong to the nerve-cells of the first type (Deiters's). There is another kind of cell, the nerve-process of which rapidly divides and remains within the gray substance. Because they do not pass beyond the gray substance these elements are named *internal cells;* they occur

* The nerve-processes coming from the vesicular column of Clarke do not divide in the white substance, but turn cranialward and proceed to the cerebellum. The nerve-processes of still other column-cells enter the white substance, and there *without* dividing, turn upward or downward. Under the name of "plurifunicular cells" column-cells have been described, the nerve-process of which divides in the gray substance into two or three branches and continues in as many fibers in different columns.

in the posterior columns, where their terminal ramification spreads out either on the same or on the opposite half of the spinal cord. They are nerve-cells of the second type (Golgi's) (Fig. 96).

The *nerve-fibers* that enter from the anterior and lateral columns partly arise from the medullated collateral fibers and ends of the nerve-processes of the column-cells, partly from the nerve-processes (likewise invested by a medullary sheath) that come from the brain.* In

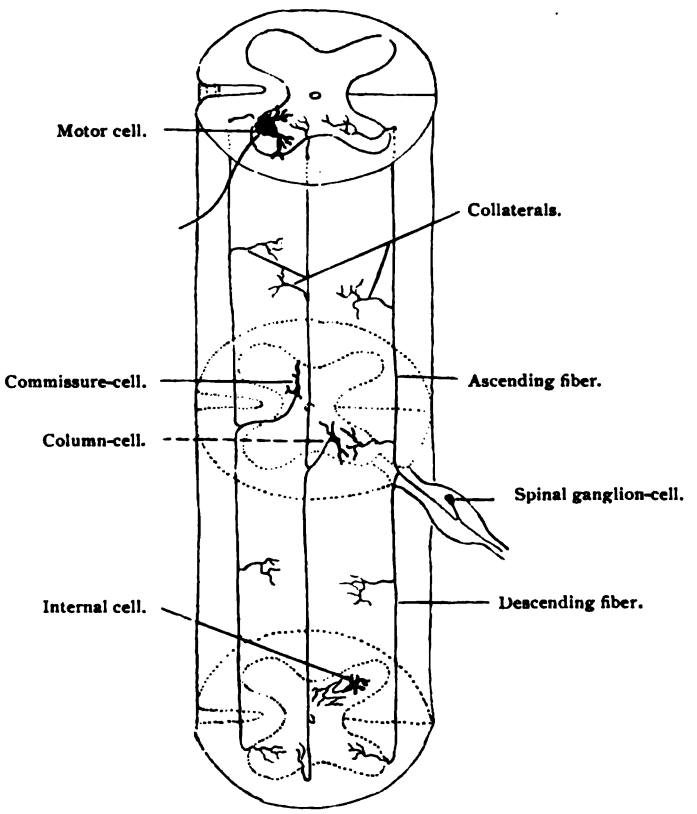



FIG. 96.—SCHEME SHOWING THE LOCATION AND RAMIFICATION OF THE NERVE-CELLS, ALSO OF THE POSTERIOR NERVE-ROOTS OF THE SPINAL CORD.

addition there are medullated nerve-fibers of the posterior (dorsal) roots which originate in the centripetal processes of the cells of the spinal ganglia. These posterior root-fibers enter the spinal cord in two groups, a lateral, which runs in the zona terminalis, and a larger median, which runs in the posterior column. These fibers do not directly enter the gray

* For an account of the exact course of these fibers the student is referred to special text-books.

substance, but each first divides Y-shape into an ascending and descending stem-fiber (Fig. 97), from which numerous collateral fibers diverge at right angles. These now enter the gray substance * and with their tufts of terminal fibrils distribute themselves over nearly every point of the same. One set terminates principally in the summit of the posterior horn; these take their origin in the lateral root-fiber group and form a very fine-fibered dense plexus, that also partly lies in the substantia gelatinosa (Fig. 98, *c*); a second set terminates in the dorsal nucleus (Fig. 98, *a*); † these originate in the median root-fiber group, as also a third set, which penetrating the middle of the substantia gelatinosa passes ventralward into the anterior horn and there radiating fan-shape surrounds the motor nerve-cells (Fig. 98, *b*); these latter very robust collateral fibers ("reflex collaterals") arise from the portion of the stem-fibers close to the point of bifurcation and form the reflex bundle.‡ Finally, a fourth, smaller set of collateral fibers passes through the posterior gray commissure to the posterior horn of the opposite side. The stem-fibers, probably only after a long course, sometimes extending into the oblongata, turn into the gray substance, where they terminate like the collaterals.

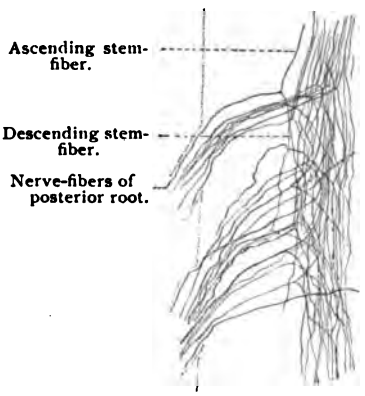



FIG. 97.—FROM A LONGITUDINAL SECTION OF THE SPINAL CORD OF A NEWBORN RAT. × 110. The section shows two posterior nerve-roots. The collateral fibers are not visible. Techn. No. 70.

The peculiarities of the substantia grisea centralis and substantia gelatinosa, which belong to the gray substance, are dependent upon the abundance of neuroglia and will be described with this.

The *white substance* consists only of medullated nerve-fibers, that do not possess a neurilemma. The fibers differ greatly in thickness; the thickest are found in the anterior columns and in the lateral parts of the

* An exception occurs in the case of some fiber-bundles which directly enter into the gelatinous subsance and partly in this or ventral thereto (in the territory of the posterior horn) divide into ascending and descending stem-fibers.

† Here the medullary sheaths extend farther than elsewhere,—that is, to the last terminal ramification.

‡ The reflex bundle and the collateral fibers of Clarke's column sink into the gray substance in a curve with the concavity lateralward and form a considerable mass easily perceived. The place at which they enter the gray substance has been named "root-entrance zone."

posterior columns; the thinnest in the median part of the posterior columns and in the lateral columns where the white touches the gray substance. In the remaining portions thick and thin fibers are intermingled. The majority of the nerve-fibers run parallel with the long axis of the spinal cord, hence in cross-sections are cut transversely. In addition there are fibers that take an oblique direction; these are found in large numbers in front of the gray commissure, where they cross at acute angles and form the white commissure (Fig. 93).

An attempt to classify the nerve-fibers according to their origin will

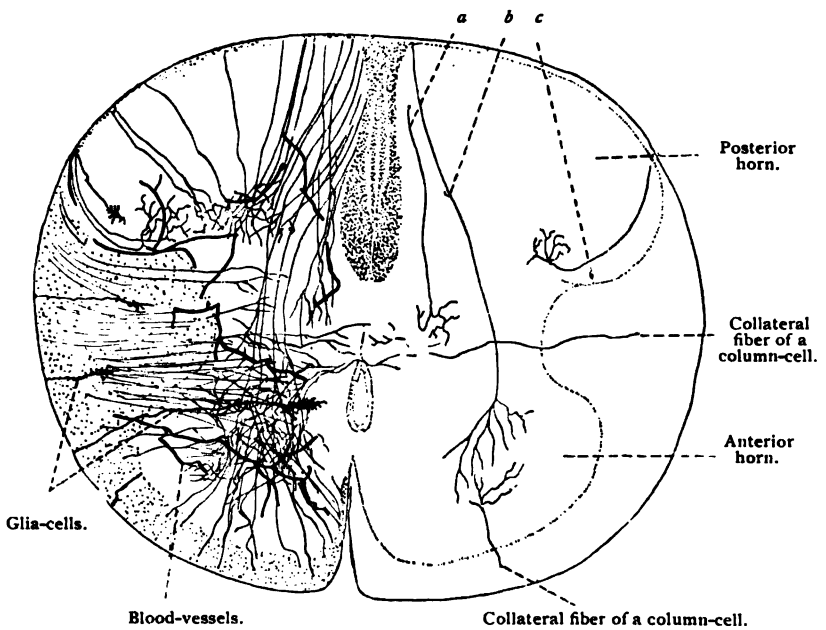



FIG. 98.—CROSS-SECTION OF THE SPINAL CORD OF A NEWBORN RAT SHOWING COLLATERAL FIBERS. × 75. In the right half only one representative of each class has been sketched. Techn. No. 70.

result as follows: 1, fibers which are continuations of the posterior root; the entire posterior column consists of posterior root-fibers, because the latter (or their stem-fibers), entering in the lumbar region, are pushed toward the median line by the fibers entering at higher levels; 2, fibers which are continuations of the column-cells; 3, fibers which are continuations of the ganglion-cells of the brain. The latter two occupy the anterior and lateral columns and are united in compact bundles (funiculi).

The *supporting framework* of the spinal cord is constructed of two genetically distinct formations: 1, *connective-tissue* extensions of the pia, which penetrate the white substance as sheaths for the blood-vessels;

this mesenchymal framework steadily grows thinner as it approaches the gray substance, into which it does not extend; 2, the *neuroglia* ("nerve-cement"), which is derived from the same embryonic anlage as the central nervous system. The neuroglia principally consists of nucleated elements, the *glia-cells*, and, possibly, of a small amount of homogeneous ground-substance. There are two kinds of glia-cells, ependymal cells and astrocytes. The *ependymal cells* in a single layer line the lumen of the central canal. In youth they are beset with cilia, their cylindrical bodies are prolonged in an extended process that in the embryo reaches to the surface of the spinal cord, where it terminates in a simple

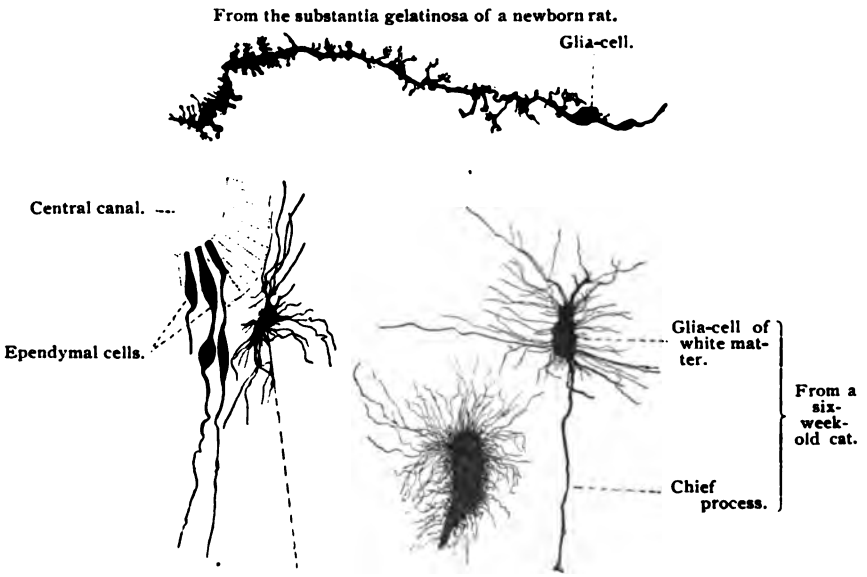



FIG. 99.—GLIA-CELLS FROM THE SPINAL CORD. × 280. Techn. No. 70.

or branched end (Fig. 99). The cells of the ependyma are phylogenetically the older; they arise also ontogenetically first, but in the further course of development undergo regression in different degrees; the long processes in particular are involved, which retain their original length, to the surface of the spinal cord, only in the region of the posterior median septum* and opposite, to the base of the anterior median fissure. In the course of development one division of the ependymal cells wanders peripheryward and becomes transformed into astrocytes. Not infrequently

* The posterior median septum consists for the greater part of processes of ependymal cells.

the central canal is completely obliterated. The *astrocytes* (Deiters's cells), in the beginning of their development lie in the gray substance; later they retreat into the white substance and then are very differently shaped. Of the numerous processes of the astrocytes one, the "chief process," frequently originates first; the others, partly finer and partly coarser "secondary" processes, arise later. Many of these cells, with much-branched processes, reach to the surface of the spinal cord, where they terminate in expanded ends * and form a distinct border on the superficial glia-zone, the "gelatinous cortical layer" or "hornspongiosa." Two varieties of the developed cells, united by many transitional forms, may be distinguished: the *mossy-cells* and the *spider-cells*. The mossy-cells possess shorter, very richly-branched processes, that not infrequently are applied to the blood-vessels; they chiefly occur in the gray substance; the spider-cells, the more usual form, have a small cell-body from which, besides short, also many longer, rigid, less-branched processes radiate (like Fig. 104); these chiefly occur in the white substance and are not apt to be confused with the ganglion-cells. By the interlacing of the numerous fine processes of neighboring glia-cells (they do not anastomose) a close web is constructed which envelops each individual nerve-fiber.

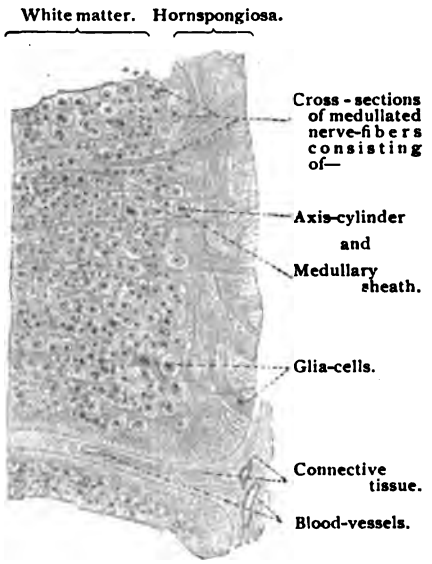



FIG. 100.—FROM A CROSS-SECTION OF THE HUMAN SPINAL CORD IN THE REGION OF THE LATERAL COLUMN. × 180. Techn. No. 69.

In the substantia grisea centralis and substantia gelatinosa the neuroglia assumes a totally different appearance. In the former the astrocytes, with their here very long, stiff, unbranched processes, are concentrically arranged in a fiber-wreath (Fig. 99). These and the cells of the ependyma are together called "central ependyma filaments." The substantia gelatinosa consists of a small number of very small ganglion-cells, the nerve-processes of which turn into the zona ter-

* These expanded ends stand close beside one another, and form a "membrana limitans meningea," that is not an independent membrane any more than the internal limiting membrane of the retina (see The Eye and its Appendages.)

minalis, of a plexus of delicate nerve-fibrils, and of nerve-fibers (collaterals) passing through; there is besides a granular substance present which has arisen by a transformation of numerous and very delicate processes of the astrocytes occurring there.

## THE BRAIN.

The brain, like the spinal cord, is composed of a white and a gray substance, which in their minute structure agree on the whole with the same substances in the cord. But the arrangement of the two substances in the brain is a much more diversified one than in the spinal cord.

The gray substance of the brain occurs in four aggregations:

(*a*) As the *cerebral cortex*, an expansion covering the entire surface of the cerebral hemispheres.

(*b*) In the form of discrete masses, which are situated in the cerebral ganglia,—the *corpora striata*, the *optic thalami*, the *corpora quadrigemina*.

(*c*) As the *lining* of the *ventricles*, which is the direct continuation of the gray substance of the spinal cord.

(*d*) As the *cerebellar cortex*, an expansion covering the surface of the cerebellum. Discrete masses also occur in the interior of the cerebellum.

All these aggregations have numerous connections with one another by means of fiber-tracts.

### The Cerebral Cortex.

In vertical sections of the cerebral cortex four zones, not sharply defined from one another, may be distinguished.

1. The *molecular zone* (neuroglia layer), the most superficial, in ordinary preparations appears finely granular or reticulated and contains, besides many glia-cells, an interlacement of medullated nerve-fibers running horizontally, the *tangential fibers* (Fig. 101). By means of Golgi's method, it may be seen that the reticulum is partly formed by the dendrites of the pyramidal cells of the second and third zones and partly by the processes of glia-cells. Besides the latter, the *cells of Cajal* occur in the molecular zone; they possess an irregularly-shaped cell-body that sends out very long processes running parallel to the surface, from one portion of which, vertically to the surface, ascending lateral twigs arise, while one or more processes penetrate into the depths of the cortex* (Fig. 102, 1).

* In animals four and even more "nerve-processes" of Cajal's cells have been described; in man the demonstration of nerve-processes has not yet been accomplished. The nervous nature of Cajal's cells is not definitely established; I am strongly inclined to regard them as glia-cells.

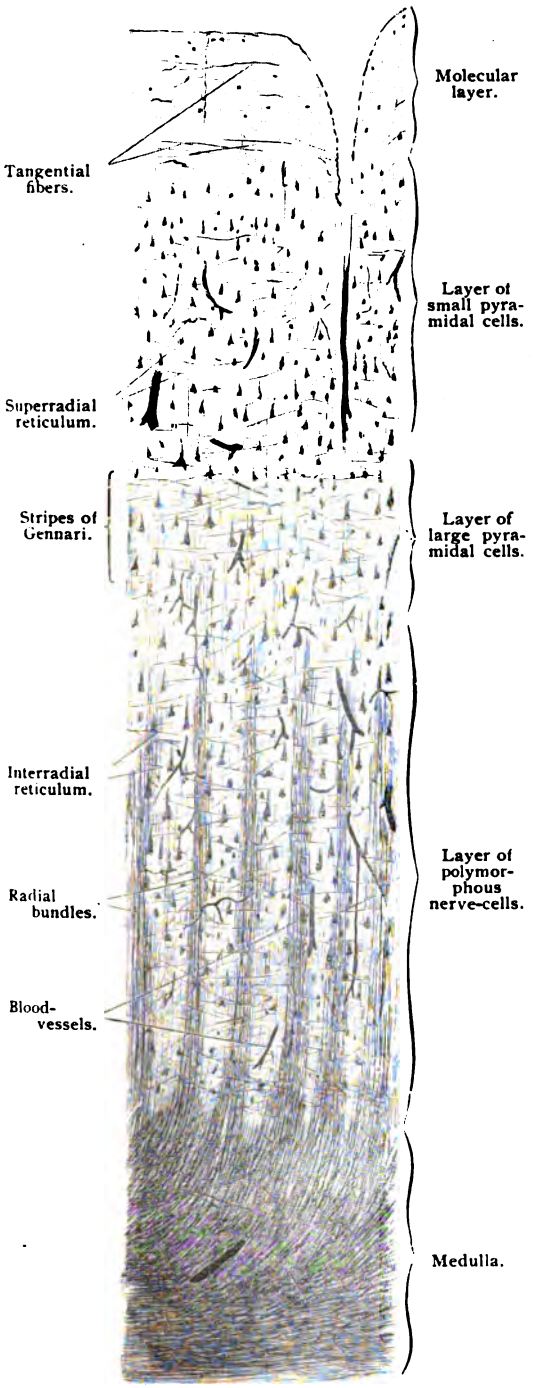



FIG. 101.—VERTICAL SECTION OF HUMAN CEREBRAL CORTEX. × 60. Techn. No. 71.

FIG 102.—SCHEME OF CEREBRAL CORTEX, sketched from specimens prepared according to Techn. No. 73, b. 1. Cell of Cajal. 2, 2'. Small pyramidal cells. 3. Large pyramidal cell. 4. Polymorphous cell. 5, 5'. Cells of the second type. 6. Nerve-fiber ending in the superficial zone; *a*, mossy-cell; *b*, spider-cell (glia-cells). The ependymal-cells are not represented.

2. The *zone of the small pyramidal cells* (Fig. 101, 102) is characterized by ganglion-cells from 10 to 12 $\mu$ in size and of a pyramidal form; the apex of the pyramidal cell is prolonged into a long ramifying protoplasmic process (dendrites),* that after giving off minute lateral twigs enters the molecular zone, where it terminates in numerous, often serrulate, branches (Fig. 102, 2); smaller dendrites spring from the lateral surfaces and from the inferior surface of the cell. The nerve-process always arises from the base and after giving off branched collateral fibers, as a rule, passes toward the white substance, there to become the axis-cylinder of one or, by division, of two nerve-fibers; occasionally, however, it turns and runs to the molecular layer, where it divides and enters the web formed by the tangential fibers (Fig. 102, 2′). The nerve-processes and the collateral fibers are enveloped in a medullated sheath.

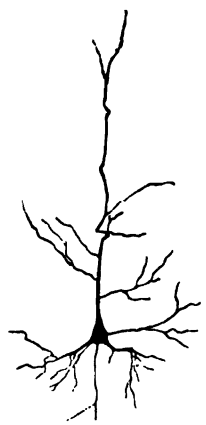



FIG. 103.—PYRAMIDAL CELL FROM A PERPENDICULAR SECTION OF THE CEREBRAL CORTEX OF ADULT MAN. × 120. The terminal branches of the dendrites running toward the molecular layer are not visible. Techn. No. 73 *b*.

3. The *zone of the large pyramidal cells* is distinguished from the preceding zone by the greater size of its elements (from 20 to 30 $\mu$), the extremely robust nerve-process, after giving off in the gray substance several collaterals always goes to the white substance (Fig. 102, 3).

4. In the *zone of the polymorphous nerve-cells* the majority of the elements are oval or polygonal; an apical dendrite is wanting, the delicate nerve-process, after sending off a number of collaterals, enters the white substance, where it passes into one or, dividing into T-branches, into two nerve-fibers (Fig. 102, 4).

In the last three zones ganglion-cells of the second type also are found. Their branched nerve-process sometimes is confined to the gray matter in the vicinity of the cell, sometimes extends to the molecular zone, where richly branched it terminates (Fig. 102, 5, 5′).

The last two zones contain numerous medullated nerve-fibers. They are partly arranged in thick "radiating" bundles, which resolve into single fibers near the zone of the small pyramidal cells (Fig. 101). These bundles are formed by the descending medullated nerve-processes

* For this reason it is difficult to determine the size of the pyramidal cells; the considerable differences in the estimated size may be referred to this gradual passage of the cell-body into the apical process.

of the large and small pyramidal cells, by thick medullated nerve-fibers of unknown origin, that ascend from the white substance toward the cortex (Fig. 102, 6), where they repeatedly divide and form the "superradial" and the tangential plexus (Fig. 102), and finally end in free branches. Another set of medullated nerve-fibers runs transversely to the radiating bundles and forms the interradial reticulum; this is somewhat condensed toward the "superradial" reticulum and thus represents the *stripes of Gennari* or *Baillarger* (Fig. 101). This and the interradial reticulum are composed of the medullated collateral fibers of the nerve-processes of the pyramidal cells.

The structure of the cerebral cortex is modified in certain localities. In the hippocampal and uncinate convolutions the tangential fibers are

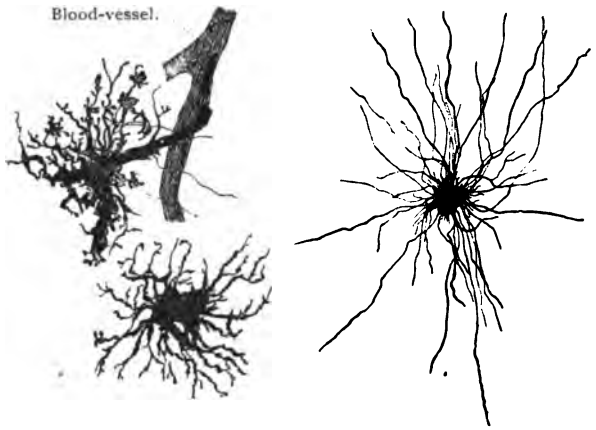



FIG. 104.—FROM SECTIONS OF THE BRAIN OF ADULT MAN. × 280. Techn. No. 73 *b*.

present in larger numbers and form an expanded net-like white layer, the substantia reticularis alba. In the vicinity of the calcarine fissure the stripes of Gennari are developed into the *bundle* of *Vicq d'Azyr*, which may be seen by the unaided eye. Greater* or lesser deviations occur in many localities, which render a classification according to the above description much more difficult.

Finally, extensions of the pia that penetrate in company with the blood-vessels participate in the construction of the cerebral cortex, as also the *neuroglia;* this like that of the spinal cord consists of ependymal cells and of astrocytes. In the embryo the peripheral processes of the

* Regarding the minute structure of the cortex of the cornu ammonis and the bulbus olfactorius, the reader is referred to special text-books.

former extend to the free surface. Of the latter two varieties are distinguished. The one variety are characterized by their small cell-body and long, rigid, little-branched processes, of which the most delicate rest like a short turf on the cell-body ; they are called *spider-cells*, and chiefly occur in the white substance. The other variety, the *mossy-cells*, have short, gnarled, richly-branched processes and are mainly found in the gray substance, where they are in intimate relation with the blood-vessels, to the walls of which they are often attached by one thicker process (Fig. 104). On the surface of the cerebral cortex there is a glia-zone formed by the ends of the thitherward extending processes of the glia-cells.

### The Cerebral Ganglia.

The gray substance of the cerebral or basal ganglia consists of ganglion-cells varying in size, medullated nerve-fibers, and neuroglia. The macroscopic variations in color depend on the different proportions in which the ganglion-cells and nerve-fibers are mingled : wealth of ganglion-cells is rendered perceptible by a dark red-brown color, profusion of nerve-fibers by a pale yellow-gray color.

### The Gray Substance of the Ventricles.

The gray substance of the ventricles extends from the floor of the fourth ventricle through the aqueduct of Sylvius into the third ventricle, to the tuber cinereum and the infundibulum. It is of especial interest as the place of origin of the cranial nerves. It is composed of neuroglia, nerve-fibers, and ganglion-cells ; the majority of the latter are multipolar and in certain localities are distinguished by their size (in the nucleus of the hypoglossal nerve), or by their peculiar form (the spherical ganglion-cells in the upper pair of the corpora quadrigemina).

An extension of the neuroglia and ependyma lining the central canal of the spinal cord lines the floor of the fourth ventricle, the aqueduct of Sylvius (aquæductus cerebri), the inner surface of the third and the lateral ventricles ; it is composed of similar elements. The columnar or cubical cells of the ependyma of the ventricles in the newborn, and in part also in the adult, possess cilia.

### The Cerebellar Cortex.

The cerebellar cortex consists of three well-defined strata of gray substance, of which the outer and the inner are macroscopically, the middle, on the contrary, only microscopically perceptible : they are from within outward, the *granule layer*, the *ganglionic layer*, and the *molecular layer*.

The *granule layer*, the innermost, is characterized by its rust color and consists of numerous strata of small cells, that by the ordinary methods exhibit a proportionately large nucleus and a very small amount of protoplasm. By the aid of Golgi's method it becomes apparent that, apart from the glia-cells, two varieties of ganglion-cells are present : *small granule-cells* and *large granule-cells.* The former are multipolar ganglion-cells with short dendrites with claw-like endings and a delicate nonmedullated nerve-process, that passes vertically into the molecular layer and there divides into two T-branches that run parallel to the surface and to the axis of the convolution and terminate in free unbranched ends (Fig. 106

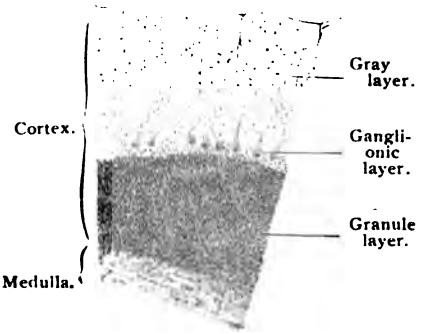



FIG. 105.—FROM A PERPENDICULAR SECTION THROUGH THE CORTEX OF THE CEREBELLUM OF ADULT MAN. × 50. Techn. No. 72.

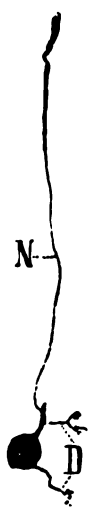



FIG. 106. — SMALL GRANULE-CELL WITH A PIECE OF THE NERVE-PROCESS, N, AND SHORT DENDRITES, D. From a section through the cortex of the cerebellum of a six-week-old cat. × 400. Techn. No. 74.

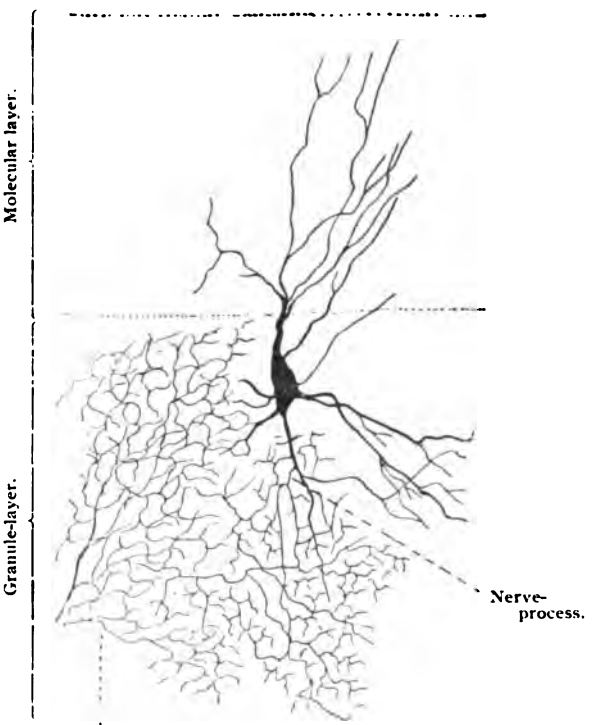



FIG. 107.—LARGE GRANULE-CELL FROM A SECTION THROUGH THE CORTEX OF THE CEREBELLUM OF A SIX-WEEK-OLD CAT. × 200. Techn. No. 74.

and 109, 1). The small-granule-cells form the chief mass of the cellular elements of the granule-layer. Less numerous are the *large granule-cells*, multipolar ganglion-cells more than twice the size of the smaller elements, the ramifying dendrites of which extend into the outermost gray layer, the nerve-process of which, running in the opposite direction, rapidly divides and terminates in a rich ramification penetrating the entire granule-layer (Fig. 107 and 109, 2).

A dense plexus of medullated nerve-fibers occurs in the granule-

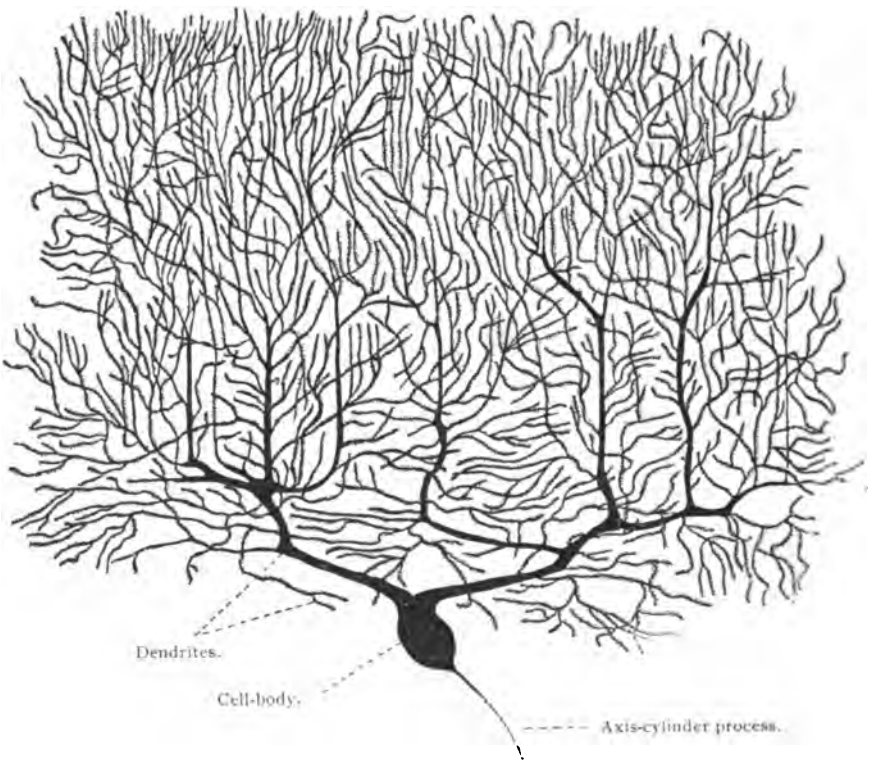



FIG. 108.—NERVE-CELL (CELL OF PURKINJE) FROM A SECTION THROUGH THE HUMAN CEREBELLAR CORTEX. × 180. Techn. No. 74.

layer (Fig. 109, 3); the greater part of the fibers come from the white substance of the cerebellum and at the boundary between the granule and the ganglionic layer form a horizontal bundle (3′) running transverse to the longitudinal axis of the convolution, from which fibers ascend into the molecular layer (3″). A small portion of this plexus is formed by the medullated nerve-processes of the cells of Purkinje.

The *middle ganglionic stratum* of the cerebellar cortex consists of a

simple layer of very large multipolar ganglion-cells, the *cells of Purkinje*. Their somewhat pear-shaped bodies send two robust dendrites into the molecular layer, where they terminate in an uncommonly rich arborization and extend to the free surface (Fig. 109, 4). The ramification does not extend in all directions, but only in planes transverse to the long axis of the convolution, therefore, the entire ramification can be seen only in transverse sections of the convolution. From the opposite pole of the

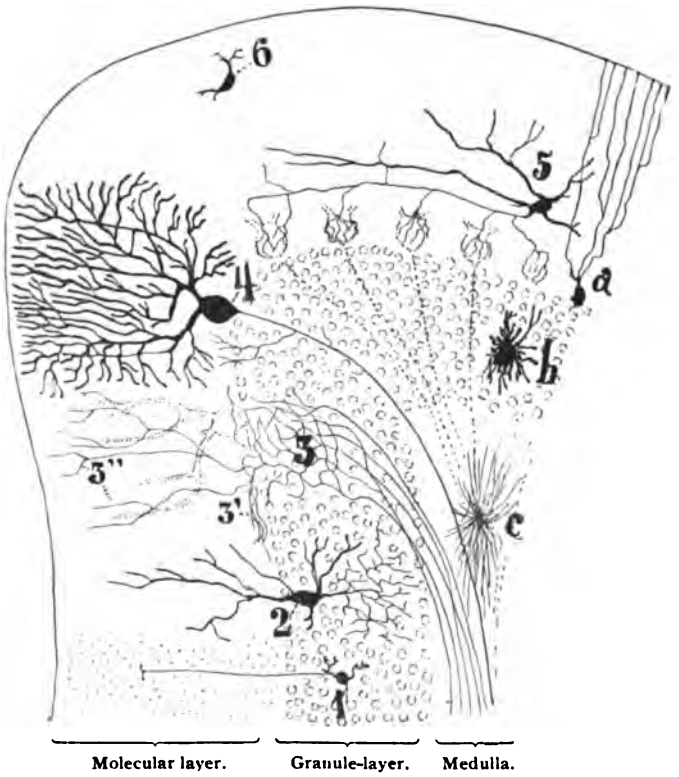



FIG. 109.—SCHEME OF THE CEREBELLAR CORTEX, sketched from specimens prepared according to Techn. No. 74. 1, Small granule-cells; 2, large granule-cells; 3, plexus of nerve-fibers; 3′, horizontal bundles; 3″, fibers of the molecular layer; 4, cell of Purkinje; 5, basket-cell; 6, small cortical cells. *a*, Glia-cells of the molecular layer; *b*, mossy-cell resembling a glia-cell; *c*, spider-cell.

cell the nerve-process arises, that immediately acquires a medullated sheath and passing through the granule-layer enters the white substance of the cerebellum; while still within the granule-layer it sends off collaterals that branch there and, in part, run back between the cells of Purkinje (Fig. 109).

The outermost *gray* or *molecular layer* is distinguished by its gray color and contains multipolar ganglion-cells, the dendrites of which

mainly extend toward the surface. Their long nerve-process runs horizontally in the transverse axis of the convolution, sends toward the surface a few collaterals, toward the interior gives off at successive intervals delicate branches the terminal ramifications of which form a basket-like network—fiber-basket—around the bodies of Purkinje's cells (Fig. 109, 5, and Fig. 110). The "basket" often also embraces the beginning of the axis-cylinder process of Purkinje's cell. These cells are called *basket-cells.**

The medullated nerve-fibers in the molecular layer are extensions of the reticulum of the granule-layer and in part pass toward the surface, where after losing the medullary sheath they terminate in free branches

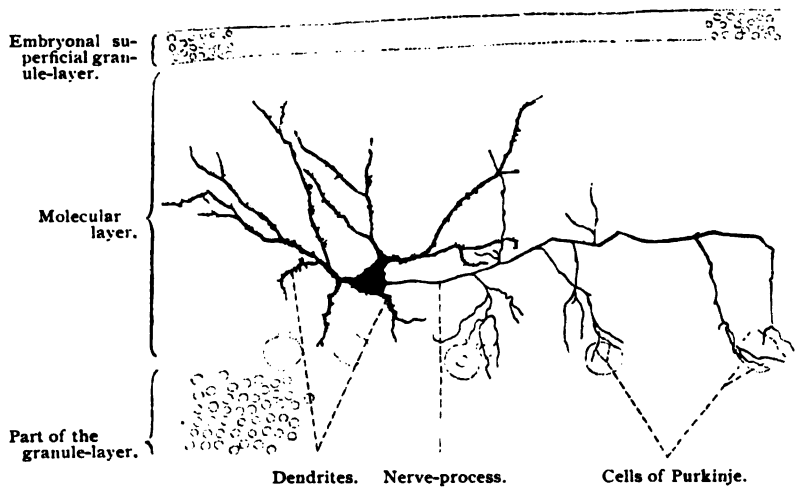



FIG. 110.—BASKET-CELL, FROM A SECTION THROUGH THE CEREBELLAR CORTEX OF A SIX-WEEK-OLD CAT. × 240. The five cells of Purkinje were not blackened but were plainly visible; only the outlines of their bodies are sketched. Techn. No. 74.

between the arborizations of the protoplasmic processes of the cells of Purkinje, in part they run horizontally between the bodies of these cells, parallel to the axis of the convolution.

The *neuroglia* of the cerebellum consists of two kinds of cells: (1) the one kind lie at the outer boundary of the granule-layer; they have a small cell-body that sends a few short processes to the interior, but many long processes in a straight course toward the free surface, where they terminate in a triangular expansion (Fig. 112, left). In this way a relatively thick peripheral glia-layer is formed. (2) The other kind are

* The so-called *small cortical cells* (Fig. 109 and Fig. 111) are also basket-cells, the processes of which have "blackened" for a shorter distance.

stellate cells resembling the mossy-cells of the cerebral cortex (Fig. 112, right); they occur in all the strata. In the white substance typical spider-cells are found.

So long as the cerebellar cortex is not fully developed it is characterized by a series of peculiarities that are wanting in the adult. In embryos and young animals there is over the as yet slightly-developed molecular layer a superficial granule-stratum; the structures in the

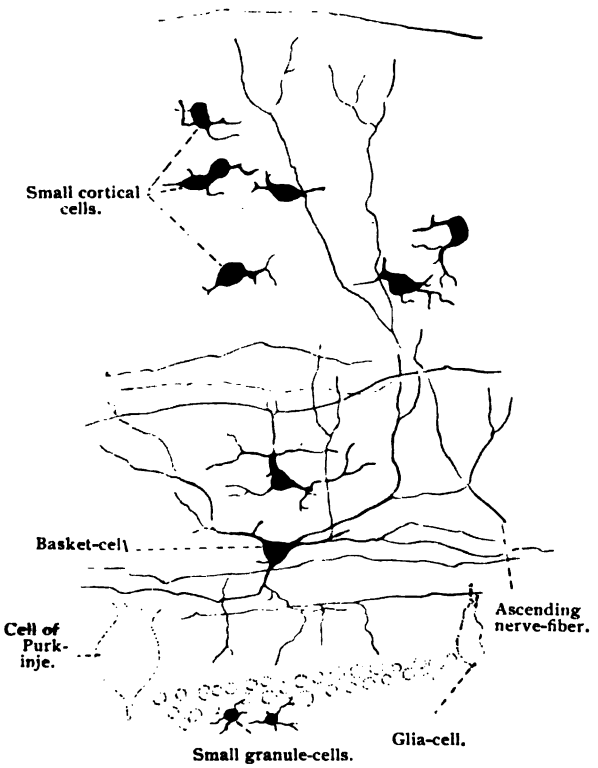



FIG. 111.—FROM A SECTION OF THE CEREBELLAR CORTEX OF ADULT MAN. × 240. The transverse lines are nerve-processes of basket-cells. The cell of Purkinje and the glia-cell were sketched in outline from another part of the specimen for the purpose of demonstrating the difference in size. Techn. No. 74.

FIG. 112. — TWO GLIA-CELLS FROM A SECTION THROUGH THE CEREBELLAR CORTEX OF ADULT MAN. × 90. On the right the body, *P*, and the dendrites, *P'*, of a cell of Purkinje are sketched in outline to demonstrate the difference between this element and the glia-cells. Techn. No. 74.

granule-layer described under the name of "mossy-fibers" are developmental forms of medullated nerve-fibers; of like significance are the "climbing plexuses," which are found in the environs of the ramifying protoplasmic processes of the cells of Purkinje.

The union of the elements of the cerebellum, as everywhere in the central nervous system, is only by contact, not by direct connection.

The *white substance*, the medulla, of the cerebrum and of the

cerebellum, apart from the elements of the supporting framework (connective tissue and neuroglia), consists throughout of medullated nerve-fibers without a neurilemma and varying in thickness from 2.5 to 7 $\mu$.

The *hypophysis cerebri* (pituitary body) is composed of two genetically different parts: (1) a *posterior small lobe* that belongs to the brain and is a continuation of the infundibulum; it contains delicate, much-branched nerve-fibers, that form a very compact plexus, and connective tissue, many blood-vessels, and cells that closely resemble bipolar or multipolar ganglion-cells, but the nature of which is still uncertain; (2) an *anterior larger lobe* derived from a diverticulum of the embryonal oral cavity; it contains gland follicles embedded in loose vascular connective tissue, the majority of which are solid and filled with clear or granular

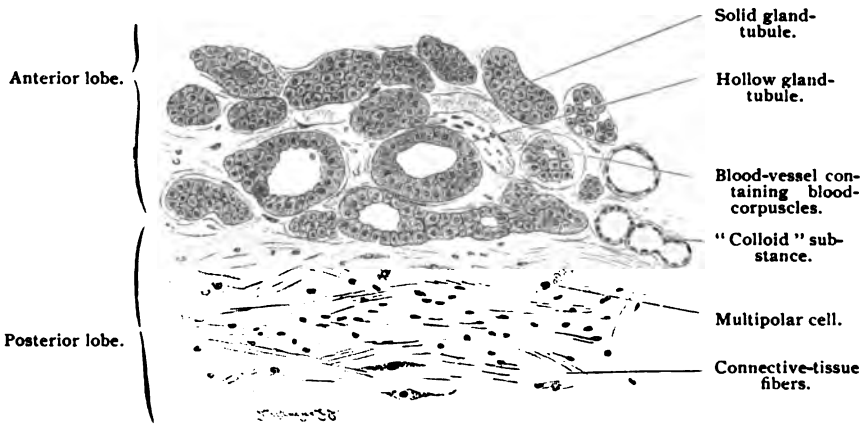



FIG. 113.—PORTION OF A HORIZONTAL SECTION OF A HUMAN PITUITARY BODY, showing the boundary-line between the anterior and posterior lobe. Two gland-tubules on the left contain each a granular epithelial-cell. × 220. Techn. No. 75.

cubical epithelial-cells (Fig. 113). Only a few of the follicles toward the border of the smaller lobe are hollow and occasionally contain a mass resembling the colloid substance of the thyroid body.

The *pineal body* (epiphysis, *corpus pineale*) is derived from a fold of the wall of the primitive brain-vesicle and consists of epithelial-cells, some of which have delicate processes, and of a connective-tissue envelope from which septa extend into the interior of the organ. Almost invariably "brain-sand" (*acervulus cerebri*) is found in the epiphysis, rounded concretions from 5 $\mu$ to 1 mm. in size, with an uneven, mulberry-like surface (Fig. 114). They are composed of an organic basis and calcium carbonate and magnesium phosphate.

Not infrequently (especially in advanced life) there occur in the

brain-substance round or discoid bodies exhibiting distinct concentric stratifiation, which stain violet on treatment with iodin and sulphuric acid, therefore are related to amylum (Fig. 115, *a*). These *corpuscula amylacea*, almost constant within the walls of the ventricles of the brain, are present in many other localities, as well in the gray as in the white substance.

FIG. 114.—ACERVULUS CEREBRI FROM THE PINEAL BODY OF A WOMAN SEVENTY YEARS OLD. × 50. Techn. No. 76.

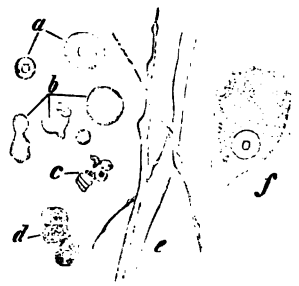



FIG. 115.—FROM A TEASED PREPARATION OF GRAY SUBSTANCE FROM THE WALL OF A VENTRICLE OF THE HUMAN BRAIN. × 240. *a*, Corpuscula amylacea; *b*, myelin drops; *c*, red blood-corpuscles; *d*, ependymal cells; *e*, medullated nerve-fibers; *f*, ganglion-cell. Techn. No. 77.

## THE MEMBRANES OF THE CENTRAL NERVOUS SYSTEM.

Two connective-tissue membranes envelop the brain and the spinal cord: the *dura* and *pia*.

The *dura* of the *spinal cord* (*dura mater spinalis*) consists of compact fibrous connective tissue and numerous elastic fibers, flat connective-tissue cells and plasma-cells (p. 79 and Fig. 120). The inner surface is covered by a simple layer of flat epithelial-cells (endothelium). It is poor in nerves and blood-vessels.

The *dura* of the *brain* (*dura mater cerebralis*) is at the same time the periosteum of the inner surface of the cranium and consists of two lamellæ: (1) an *inner*, which corresponds to the dura of the cord and is of like structure, and (2) an *outer*, which corresponds to the periosteum of the vertebral canal. It is composed of the same elements as the inner lamella, with the exception that the outer fiber-bundles are disposed in a different direction; anteriorly and laterally they extend posteriorly and medianward, like the inner fibers, which run from the anterior median region posteriorly and lateralward. The outer lamella is rich in blood-vessels, which pass from it into the cranial bones.

The *pia* of the brain and the spinal cord is a two-layered sack. The outer layer, the *arachnoid* of authors, is covered on its free surface by a

simple layer of epithelium (endothelium), and is not closely attached to the dura. The inner layer (the "pia") closely invests the surface of the brain and the spinal cord and sends vascular processes into their substance. The arachnoid and the pia are joined together by numerous lamellæ and trabeculæ extending from the inner surface of the former to the outer surface of the latter. Hernia-like evaginations occur on the outer surface of the arachnoid in certain localities, in particular near the superior longitudinal sinus, which pushing the attenuated dura before them project into the venous sinus. These are the so-called *arachnoidal granulations* (*Pacchioni*), which were long regarded as pathologic. The pia is composed of delicate connective-tissue bundles and plate-like cells, which cover the inner surface of the arachnoid and the lamellæ and trabeculæ.

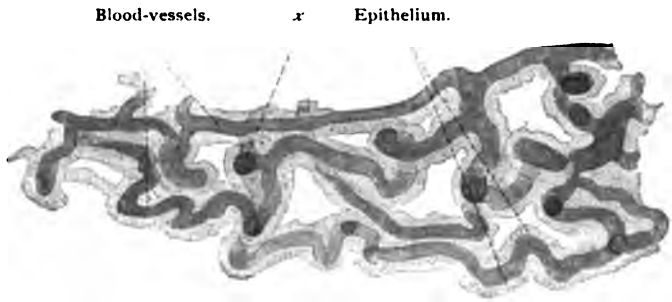



FIG. 116.—PORTION OF THE PLEXUS CHOROIDEUS OF ADULT MAN. × 80. *x*, Blood-vessel in optical cross-section. The dots in the epithelium are not nuclei, but pigment and fat-granules. Techn. No. 78 *b*.

The *telæ choroideæ* and *plexus choroidei* consist of connective tissue and numerous blood-vessels, the fine ramifications of which are united into lobules that are suspended within the ventricles. They are covered by a simple layer of cubical epithelial-cells, ciliated in the newborn, which enclose pigment-granules or oil-globules.

### THE VESSELS OF THE CENTRAL NERVOUS SYSTEM.

The *blood-vessels* of the central nervous system form a narrow-meshed capillary network in the gray, a wide-meshed network in the white substance, which are everywhere connected with each other. The capillaries of the cerebral cortex open into veins that do not take their origin in the cortex, but beneath in the white substance and from there traverse the cortex and go to the veins lying in the pia. Therefore the blood in the capillaries must traverse the entire cortex before it empties into the veins. All the blood-vessels possess a second so-called adventitial sheath, which often consists of only a simple stratum of endothelial

cells. The walls of the intradural venous sinuses are formed by a simple endothelial membrane.

The *Lymph-channels.*—Between the dura and the arachnoid there is a deep capillary cleft or fissure, the *subdural space*, which communicates with the deep cervical lymph-vessels and lymph-nodes (at least in the rabbit and the dog), with the lymph-channels of the peripheral nerves, with the lymph-vessels of the nasal mucous membrane, with the smaller clefts (juice-canals) in the dura, and finally, round the arachnoidal villi, with the intradural venous sinuses. The fluid in the subdural space is very scanty.

The *subarachnoidal space*, that between the two layers of the pia (or arachnoid and pia), communicates with the "juice-channels" of the peripheral nerves, the lymph-vessels of the nasal mucous membrane, the interior of the ventricles of the brain and of the central canal of the spinal cord. The fluid in the subarachnoid space is very abundant; it is called the *cerebro-spinal fluid.*

The spaces occurring within the adventitial sheath of the blood-vessels can be injected from the subarachnoidal space. They are called *adventitial lymph-spaces.*

The spaces filled only by injecting the brain-substance itself cannot be included in the system of lymphatic channels. These spaces occur as *pericellular spaces* surrounding the larger ganglion-cells of the cerebral cortex, also many glia-cells; as *perivascular spaces* of the blood-vessels, that formed by the adventitial sheath excepted; and between the pia and the cerebrum, as the *epicerebral space.* These may be regarded as an independent juice-canal system.

## 2. THE PERIPHERAL NERVOUS SYSTEM.

### The Nerves.

The *cerebro-spinal nerve-trunks* chiefly consist of medullated nerve-fibers varying in thickness and of only a few gray nerve-fibers; therefore by reflected light they appear white. Their mode of union agrees in many respects with that of the striated muscle-fibers. A sheath formed of loose connective tissue and elastic fibers, often containing clusters of fat-cells, surrounds the entire nerve. It is called the *epineurium* (Fig. 117). Extensions of the epineurium in the interior of the nerve surround the (so-called secondary) nerve-fiber bundles (funiculi), of which each is enveloped by the concentrically-lamellated connective-tissue *perineurium.* From the latter connective-tissue septa extend into the

interior of the secondary nerve-fiber bundles; they constitute the *endoneurium*. Finally, delicate lamellæ from the endoneurium, the *fiber sheaths*, corresponding to the perimysium of the single muscle-fiber, surround each individual nerve-fiber. These sheaths are in direct con-

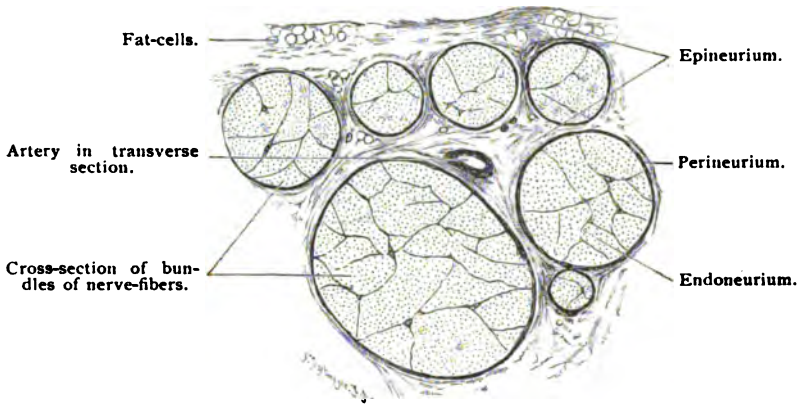



FIG. 117.—PORTION OF CROSS-SECTION OF HUMAN MEDIAN NERVE. × 20. Techn. No. 79.

nection with processes of the dura and the pia. Perineurium and endoneurium are composed of bundles of fibro-elastic tissue and of a number of concentric lamellæ; each lamella is formed by a simple layer of flattened connective-tissue cells, the outlines of which can be demon-

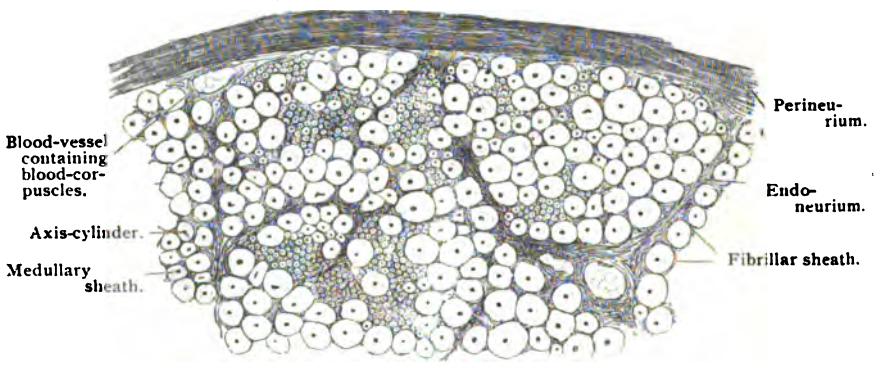



FIG. 118.—PORTION OF CROSS-SECTION OF HUMAN MEDIAN NERVE. × 220. Techn. No. 79.

strated by silver staining. The fiber-sheaths, in addition to delicate connective-tissue bundles, also consist of plate-like cells. The nerve-fiber bundle not infrequently divides; a variable number of nerve-fibers branch off from one funiculus to join another funiculus, the result of

which is a narrow-angled plexus of nerve-fiber bundles. Division of the nerve-fibers does not occur until at the periphery.

The *sympathetic nerve-trunks* are partly whiter and partly grayer in color, depending upon the greater or lesser number of fine medullated nerve-fibers present; for example, the splanchnic nerves contain many medullated nerve-fibers, while the gray branches of the abdominal and pelvic plexuses contain only a very few of the thinnest medullated and, on the other hand, numerous nonmedullated nerve-fibers. One portion of the medullated nerve-fibers are continuations of the spinal nerves, another portion are nerve-processes of sympathetic nerve-cells; long dendrites of sympathetic nerve-cells occasionally occur in the course of the sympathetic nerves. The nerve-fibers are held together and grouped into bundles by connective tissue.

The blood-vessels run lengthwise within the epineurium and form capillary networks with elongated meshes that are supported by the perineurium and endoneurium.

The lymph-channels occur in the capillary clefts between the lamellæ of the perineurium and between the individual nerve-fibers, so that each nerve-fiber is bathed in lymph. They are in communication with the subdural and the subarachnoidal space, but not with the lymph-vessels accompanying the nerve-trunk.

## The Ganglia.

Ganglia are groups of nerve-cells intercalated in the course of the peripheral nerves. They are usually macroscopically visible. All ganglia consist of small bundles of nerve-fibers between which lie ganglion-cells partly arranged in rounded groups, partly in longitudinal rows (Fig. 119). A connective-tissue capsule, an extension of the perineurium, covers the outer surface of the ganglion and sends into the interior delicate processes for the support of the nerve-fibers and ganglion-cells. The ganglia are very rich in blood-vessels, the capillaries of which surround the individual cells. Respecting the minute structure, differences exist between the spinal ganglia and the sympathetic ganglia.

The cells of the spinal ganglia are bipolar in the embryo; the processes spring from opposite poles of the cell. In the course of development the two processes gradually approach each other by attenuation of the portion of the cell-body from which they arise, until finally they proceed from a common stalk; the cell thus becomes unipolar.*

* In amphibians and birds, also in the embryos of mammals, isolated multipolar ganglion-cells occur; but their dendrites are short and only slightly branched.

The process of the adult cell receives a medullary sheath and a neurilemma very near to its exit from the cell, and after a short course invariably divides at a node of Ranvier into two T- or Y-branches. One branch, the cellulipetal, passes as the axis-cylinder of a sensory nerve-fiber to the periphery of the body; the other, the cellulifugal, usually the smaller branch, enters the spinal cord as a constituent of a posterior nerve-root and terminates in free branches in the gray substance. Thus each spinal ganglion-cell, by its undivided process, is in a measure intercalated in the course of a sensory nerve-fiber. The cells of the spinal ganglia are large, round, often pigmented, their vesicular nucleus contains a conspicuous nucleolus. Each cell is enveloped in a nucleated

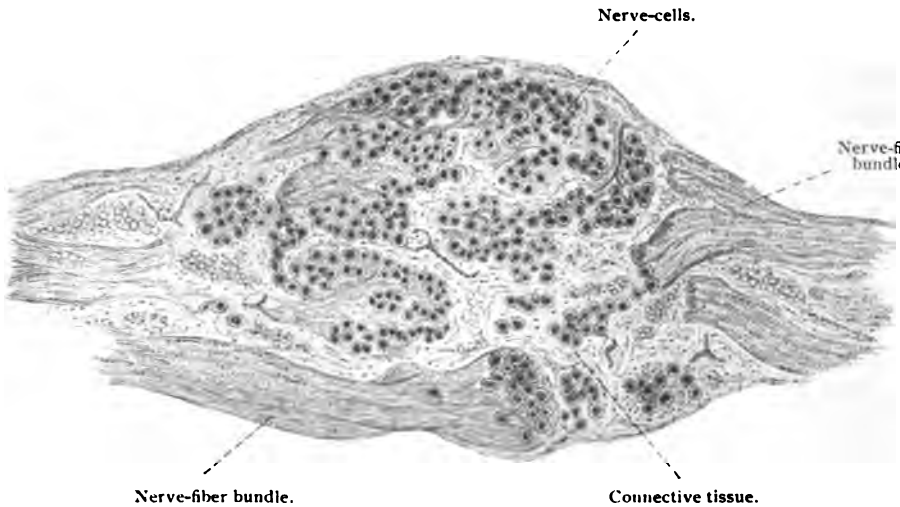



FIG. 119.—LONGITUDINAL SECTION OF THE SPINAL GANGLION OF A CALF. × 20. (Schaper.) Techn. No. 80.

capsule (Fig. 120) which consists of concentric strata of flattened connective-tissue cells and is prolonged on to the process of the cell as the *fiber-sheath.** Gray nerve-fibers from the sympathetic ganglia occur in the spinal ganglia, which branch and form a plexus within the connective-tissue capsule of the spinal ganglion-cells.

Other ganglia possessing the same structure as the spinal ganglia are: The Gasserian, the jugular ganglia, the plexus nodosus of the

* Whether any nerve-fibers pass through the spinal ganglia that do not enter into relation with the ganglion-cells is uncertain. In young embryo chicks such fibers have been seen coming from the cells of the anterior horns, but they have not been found in mammals.

vagus, the petrosal and the geniculate ganglia; the ganglia of the auditory nerve (ganglia nervi cochleæ et nervi vestibuli) contain bipolar cells.

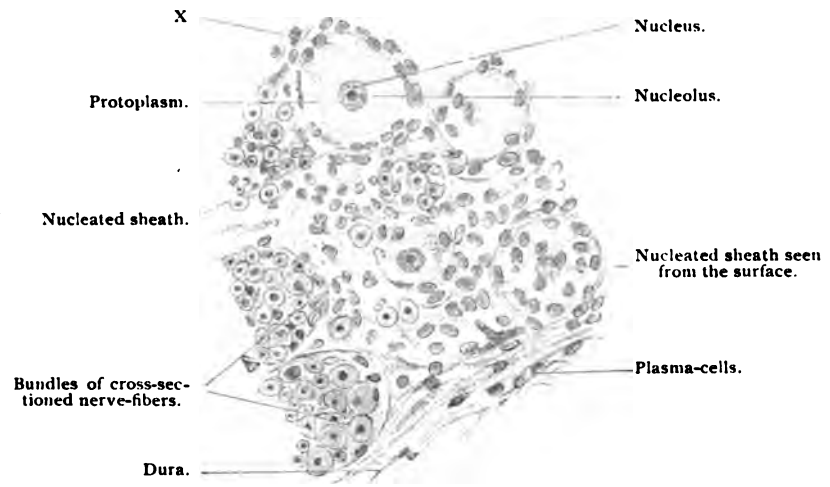



FIG. 120.—FROM A CROSS-SECTION OF THE GASSERIAN GANGLION OF MAN. × 240. The cell-processes cannot be seen in such sections. At X the protoplasm of the ganglion-cell has retracted and simulates a process. In the axis of the transversely cut nerve-fibers the axis-cylinders are seen in section. Techn. No. 80.

The *sympathetic ganglia* consist of nerve-fibers and of small, often pigmented cells, likewise surrounded by a nucleated connective-tissue

FIG. 121.—PORTION OF A SECTION OF THE SUPERIOR CERVICAL GANGLION OF MAN. × 240. Techn. No. 81.

capsule, that possess one or two nuclei (two in the rabbit and the guinea-pig). The cells are multipolar; * their branched dendrites press between

* The nerve-cells of the sympathetic ganglia of fishes are bipolar; in amphibians, perhaps also in mammals, ganglion-cells occur, of which the single process with T-branches is embraced by a "spiral fiber," that surrounds the ganglion-cell in a ramification of free branches, similar to the cells of the spinal ganglia.

the neighboring nerve-cells through to the periphery of the ganglion, where they form a "universal * peripheral plexus;" other dendrites even extend into the neighboring ganglia, where they terminate in like manner. Their nerve-process passes directly into a delicate nerve-fiber, that either becomes medullated in varying length or remains non-medullated. These fibers are for the greater part motor and terminate—often after running an extended course—on smooth muscle-fibers (p. 196); one portion terminates in free endings in the mucous membrane; the terminal ramifications of another portion surround the nerve-cells. The dense terminal ramifications of the medullated nerve-fibers coming from the motor spinal nerves form plexuses which in part lie between the nerve-cells and in part pierce their connective-tissue capsules and surround the cells themselves.

To the sympathetic ganglia belong the ganglion ciliaire, sphenopalatinum, oticum, and submaxillaire.

## THE PERIPHERAL NERVE-ENDINGS.

### Terminations of Sensory Nerves.

The peripheral terminal branches of the sensory nerves either are distributed naked, as *free endings*, or they are enclosed by epithelial or connective-tissue cells with which they form *special endings* (terminal corpuscles, end-organs).†

The *free nerve-endings* occur in such a manner that the nerve-fiber loses its medullated sheath, divides repeatedly and forms a plexus of primitive fibrils that terminate in pointed or club-shaped ends. These endings chiefly occur in stratified epithelium. They have been demonstrated with certainty in the cornea, in the oral mucous membrane (see The Taste-buds), and in the deeper strata of the epidermis. In the latter cells provided with long, branched processes, the *cells of Langerhans*, occur; these were formerly regarded as migrated wandering cells from the corium and it is possible that a few of them may have such an origin; but the majority are derived from ordinary epithelial-cells; all the transitional forms, from the typical epithelial-cells to the stellate bodies in question, may be found.

Sensory nerves have been found also in the muscles. The nerve-fibers lose their medullated sheath and invested only by the nerve-nuclei

* The expression "universal" is used in contradistinction to the statement of R. y Cajal, according to which each individual nerve-cell is surrounded by a basket formed of dendrites.

† The nerve-endings of the *neuro-epithelial cells* are described in the chapters on the special-sense organs.

divide dichotomously into numerous delicate fibrillæ that extend lengthwise between the muscle-fibers and terminate in free endings.

The *terminal corpuscles* or *special endings* may be divided into two

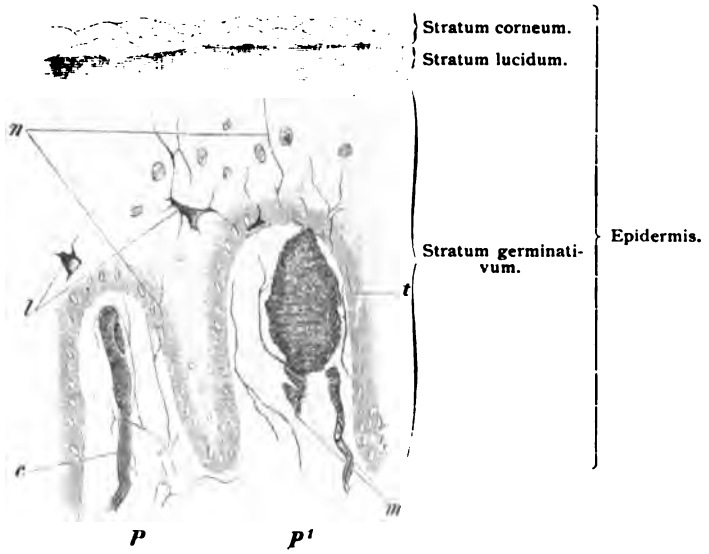



FIG. 122.—VERTICAL SECTION OF THE SKIN OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OF AGE. × 200. The cell-nuclei of the stratum germinativum are distinct only in the deepest layer. *l*, Cells of Langerhans; *n*, intra-epithelial nerve-fibers. *P*, *P*¹, two papillæ of the corium; *P* contains a capillary loop, *c*, of which only one limb is visible; *P*¹ contains a tactile corpuscle, *t*, with two approaching medullated nerve-fibers, *m*. Both papillæ contain nonmedullated nerve-fibers. Techn. No. 82.

groups: *tactile cells* and *end-bulbs*. In the tactile cells the nerve-fiber terminates in relation with one or two cells; in end-bulbs it terminates in the interior of a finely-granular body, the so-called inner bulb.

## TACTILE CELLS.

The tactile cells may be either *simple* or *compound*. The *simple tactile cells* are oval, nucleated bodies measuring from 6 to 12 $\mu$ (Fig. 123),

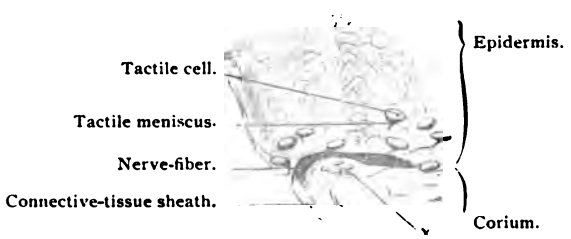



FIG. 123.—FROM A VERTICAL SECTION OF THE SKIN OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OLD. × 240. The outlines of the cells and nuclei of the epidermis can only be indistinctly seen. *x*, Tactile cells in the corium, resting upon the ramifications of a delicate nerve-fiber. Techn. No. 82.

which occur in the deepest strata of the epidermis and the outer root-sheath of the hairs or in the adjacent portions of the corium. The tactile cells rest on the *tactile meniscus*, a crescentic expansion of a non-medullated nerve-fiber.

The *compound tactile cells* (Grandry's and Merkel's corpuscles) consist of two or more somewhat flattened cells, each larger than a simple tactile cell (15 $\mu$ deep and 50 $\mu$ wide) that contain a vesicular nucleus. A medullated nerve-fiber approaches the compound tactile cells (Fig. 124) and the forks of the divided axis-cylinder clasp a flat disc, the

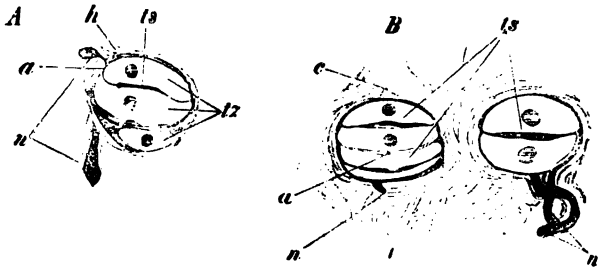



FIG. 124.—FROM VERTICAL SECTIONS OF THE SKIN OF THE BEAK OF A GOOSE. × 240. *A*. Compound tactile cell (simple tactile corpuscle), cut parallel to the course of the entering nerve-fiber: *n*, medullated nerve-fiber only partially met by the section; *a*, axis-cylinder: its division is here, in profile, invisible; *ts*, tactile discs cut vertically; *h*, connective-tissue sheath; *tz*, tactile cells. *B*. Two compound tactile cells cut transversely to the plane of the entering nerve-fiber: 1. "Simple tactile corpuscle," consisting of four tactile cells; 2, twin tactile cells; *ts*, tactile discs; *a*, axis-cylinders in transverse section, before dividing; *n*, medullated nerve-fibers; *c*, corium. Techn. No. 83.

*tactile disc* (*ts*), that lies between two flattened cells (*tz*). The nerve-fiber loses its medullated sheath at the point of entrance and the neurilemma becomes fused with the connective tissue of the capsule (*h*) enveloping the tactile cells. The compound forms consisting of two tactile cells are named twin tactile cells (*B* 2), those containing three or four tactile cells, "simple tactile corpuscles." The compound tactile cells have only been found in the epidermis of the beak and in the tongue of birds, especially in web-footed birds; they are almost exclusively situated in the uppermost strata of the corium.

### END-BULBS.

The end-bulbs are spherical or oval bodies in the interior of which nerve-fibers terminate, sometimes in a simple, sometimes in a branched ending. There are various forms of end bulbs.

The so-called *cylindrical end-bulbs*, the simplest form, chiefly consist of a modified extension of the entering nerve-fiber and comprise three parts,—the *axis-cylinder*, the *inner bulb*, and the *capsule*. The capsule is composed of flattened connective-tissue cells, the continuation of the

fiber-sheath. The inner bulb is a finely-granular mass which exhibits concentric stratification and a few nuclei at the periphery. The nerve-fiber loses its medullary sheath on entering the inner bulb, in which the axis-cylinder ascends as a flat band and terminates at the upper pole in a free or club-shaped ending. The cylindrical end-bulbs are found in the tunica propria of mucous membranes; for example, in the scleral conjunctiva of mammals and in the oral mucous membrane.

The *lamellar corpuscles* (Vater, Pacini) are transparent, elliptical structures, from 2 to 3 mm. long and 1 to 2 mm. thick, and, like the cylindrical end-bulbs, consist of a capsule, an inner bulb, and an axis-cylinder. The latter possess the same structure as in the end-bulbs,*

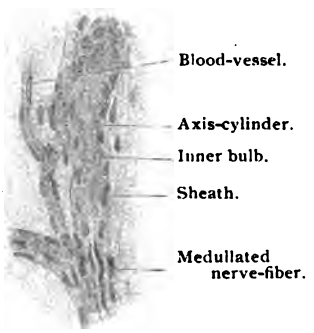



FIG. 125.—CYLINDRICAL END-BULB FROM THE CONJUNCTIVA OF A CALF. × 240. Techn. No. 84.

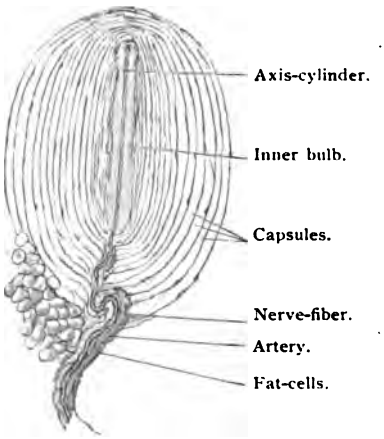



FIG. 126.—SMALL LAMELLAR CORPUSCLE FROM THE MESENTERY OF A CAT. × 50. The cells lining the capsules may be recognized by their prominent nuclei. The medulla of the nerve-fiber may be traced to the inner bulb. Techn. No. 85.

but the capsule is differently formed. It consists of a large number of concentric capsules, or lamellæ, each lined by a simple layer of endothelioid cells and separated from neighboring lamellæ by a serous fluid. Each lamella consists of an outer transverse and an inner longitudinal layer of connective-tissue fibers. As the capsule of the end-bulbs, so also these capsules originate from the connective-tissue sheath of the entering nerve-fiber. They are the smaller the nearer they lie to the inner bulb. Along the course by which the entering nerve passes to the inner bulb the lamellæ are not infrequently united by a longitudinal

* In the lamellar corpuscles the axis-cylinder not infrequently is forked at its extremity or it breaks up into several twisted interlacing twigs.

strand of tissue, the *interlamellar ligament.* A small artery accompanies the nerve-fiber into the interior of the corpuscle, which breaks up into a capillary network lying between the peripheral lamellæ.

The lamellar corpuscles partly occur in superficial situations, abundantly in the subcutaneous connective-tissue of the palm of the hand and the sole of the foot, more sparingly in other areas of the skin, in the nipples, in the territory of the pudendal nerve, partly in deeper situations, in the vicinity of the joints, on the nerves of the periosteum and the bones, and in the neighborhood of the pancreas, in the mesentery.

The corpuscles of Herbst and Key-Retzius, occurring in birds, are also lamellar corpuscles; they only differ in being much smaller and in possessing a double row of longitudinally-disposed nuclei in the inner bulb.

The *genital nerve-corpuscles* of the lower mammals and of man are spherical or oval forms (from 0.06 mm. to 0.4 mm. long), and consist of a finely-granular, nonnucleated inner bulb enveloped in a connective-tissue capsule containing cells rich in protoplasm. The approaching medullated nerve-fibers make several turns around the corpuscle, lose their medulla and divide, while fiber-sheath and neurilemma pass into the capsule; the naked axis-cylinders penetrate the inner bulb at different points, undergo rapid division and form a dense plexus of fibrils with varicose enlargements. In imperfect staining the varicosities simulate club-shaped endings. Each plexus is joined to neighboring plexuses by delicate nerve filaments.

FIG. 127.—TACTILE CORPUSCLE FROM A PERPENDICULAR SECTION OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OLD. × 560. *n*, Medullated nerve-fibers; *e*, varicosities; *h*, connective-tissue sheath. The nuclei are invisible. Techn. No. 82.

The *genital corpuscles* lie in the depths of the corium at various distances from the papillary stratum; in the papillæ only smaller corpuscles, resembling the "spherical end-bulbs," are found. The largest number, from one to four to the square millimeter, occurs in the glans penis and in the clitoris. The so-called *spherical end-bulbs* (they are sometimes round, sometimes oval) have a similar structure; they are found in the conjunctiva and in the adjoining portions of the cornea of man, and possess a greatest diameter of 0.02 to 0.1 mm. The *articular nerve-corpuscles* belong to the same category.

The *tactile corpuscles* (Wagner's and Meissner's corpuscles) are elliptical structures, from 40 to 100 μ long and 30 to 60 μ broad, which are characterized by cross-markings. They possess a connective-tissue capsule (Fig. 127, *h*) with flattened cells, the boundaries of which, as

well as their transversely-placed nuclei, produce the cross-striations just mentioned. One or two medullated nerve-fibers approach each tactile corpuscle (Fig. 127, *n*), make transverse tours encircling the lower pole of the corpuscle, part with their neurilemma and fiber-sheath, which blend with the tissue of the capsule, then lose their medullary sheath, and as naked axis-cylinders enter into a granular substance corresponding to an inner bulb ; there they form a complicated plexus beset with varicosities (*c*).* These tactile corpuscles lie in the papillæ of the corium and are most numerous (twenty-three to one square millimeter) on the palm of the hand, on the finger-tips, and on the sole of the foot.

### Terminations of the Motor Nerves.

The small nerve-trunks supplying striated muscle divide into branches, these subdivide into twigs (nerve-fiber bundles) that anastomose with one another and form a plexus, the *intermuscular plexus*. In the compass of this plexus the medullated nerve-fibers undergo numerous divisions, so that the number of nerve-fibers is considerably increased.

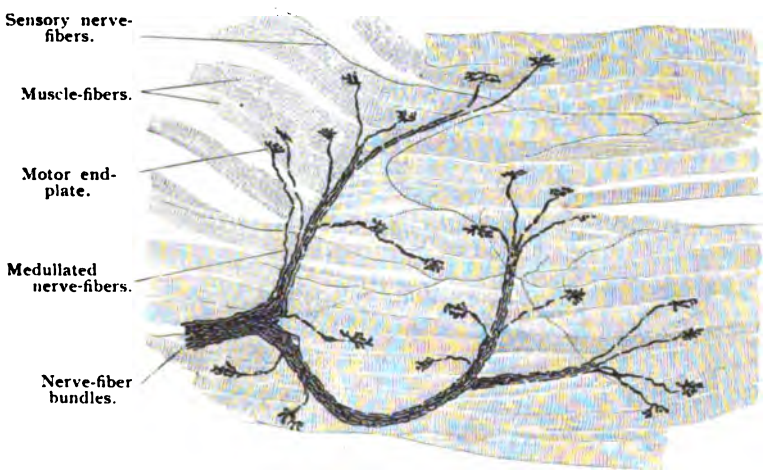



Fig. 128.—Motor Nerve-endings of Intercostal Muscle-fibers of a Rabbit. × 150. Techn. No. 86 *a*.

From the small bundles of the plexus single delicate nerve-fibers spring, each one of which finally connects with a muscle-fiber. At the point where the nerve-fiber comes into contact with the muscle-fiber it loses its medullated sheath, the axis-cylinder breaks up into a number of slightly-

* In imperfect staining the varicosities simulate club-shaped endings.

tortuous terminal branches with bulbous, swollen extremities, which form the so-called *motor end-plate* and rest upon a rounded, finely-granular disc (*sole-plate*) containing numerous vesicular nuclei. Each muscle-fiber possesses at least one motor end-plate; whether they lie upon or under the sarcolemma is not yet definitely determined.

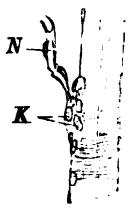



FIG. 129.—MOTOR NERVE-ENDING IN A FIBER OF AN OCULAR MUSCLE OF RABBIT. × 240. *N*, Medullated nerve-fiber; *K*, nuclei of the disc. The transverse striæ are distinct only in the lower half of the muscle-fiber. Techn. No. 86 *b*.

The nerves supplying the smooth muscles form a plexus from which bundles of non-medullated nerve-fibers arise; the latter divide repeatedly and form several networks, from which spring the most delicate nerve-fibers. They apply themselves to the smooth muscle-fibers and often are slightly thickened at the point of contact.

## THE SUPRARENAL BODY.

The description of the suprarenal body with the organs of the nervous system is warranted by the profusion of its nervous elements, by its relations to the central nervous system, as established by experiment, as well as by the facts of comparative anatomy.

Each suprarenal body consists of a cellular parenchyma and a connective-tissue capsule, which sends delicate processes into the interior of the organ. The parenchyma consists of an outer stratum, the *cortex*, which surrounds an inner mass, the *medulla*, on all sides. The *cortex* in the fresh state is of a yellow color and is composed of groups of cells about 15 $\mu$ in size, rounded in shape, that possess a coarsely-granular protoplasm, sometimes containing fat particles, and a clear nucleus. In the outer zone of the cortex the cells are grouped in oval masses; in the middle zone they are arranged in cylindrical columns, while in the innermost zone the anastomosing cords of cells lie irregularly scattered in a reticulum of connective-tissue; the cells of the innermost zone are characterized by their pigmentation. According to the described arrangement the cortex is divided as follows: 1, the *zona glomerulosa;* 2, the *zona fasciculata;* 3, the *zona reticularis.* The *medulla* in the fresh state is sometimes lighter, sometimes darker than the cortex; it consists of polygonal cells * possessing a finely-granular protoplasm and a clear

* Whether these cells are gland-cells that discharge their secretion into the veins is a question that demands further investigation.

nucleus. They are arranged in spherical groups or oval cords joined in an irregular network.

The *arteries* divide in the connective-tissue capsule into numerous small branches, that penetrate the cortex and there form a long-meshed capillary network, which passes into the medullary substance where the meshes are round. From the latter the veins proceed, of which the larger are accompanied by longitudinal strands of smooth muscle-fibers. While still within the medulla the veins unite and form the chief vein, the suprarenal vein.

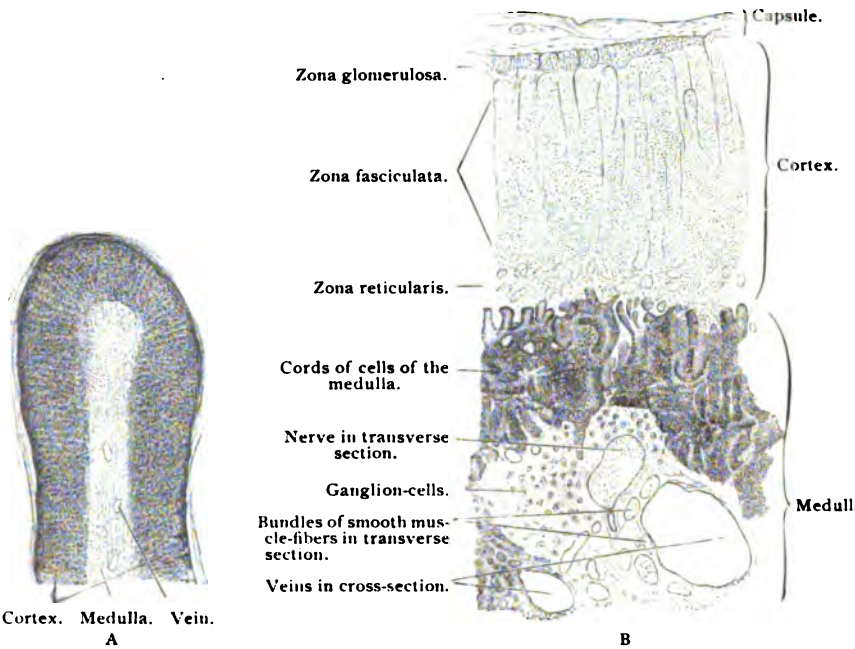



FIG. 130.—A. SECTION OF THE SUPRARENAL BODY OF A CHILD. ✓ 15. Techn. No. 87. B. SECTION OF A HUMAN SUPRARENAL BODY. ✓ 50. Techn. No. 89.

The numerous, chiefly nonmedullated *nerves* (in man about 33 small trunks) come principally from the celiac plexus and pass with the arteries through capsule and cortex to the interior of the medulla. During this course they give off a few twigs to the capsule, that form a plexus there; from this delicate branches descend into the cortex between the cell-groups of the zona glomerulosa and fasciculata, which terminate on the surface of the cell-clusters, without penetrating between the individual cells. Richer is the nerve-plexus of the zona reticularis, which originates by the branching of fibers that descend straight through the cortex; it also surrounds only cell-groups. In the medullary substance the nerve-plexus is extraordinarily dense; each individual cell is sur-

rounded by nerve-fibers. In the medullary substance, seldom in the cortex, groups of sympathetic ganglion-cells occur. Some of the nerves terminate in the walls of the blood-vessels.

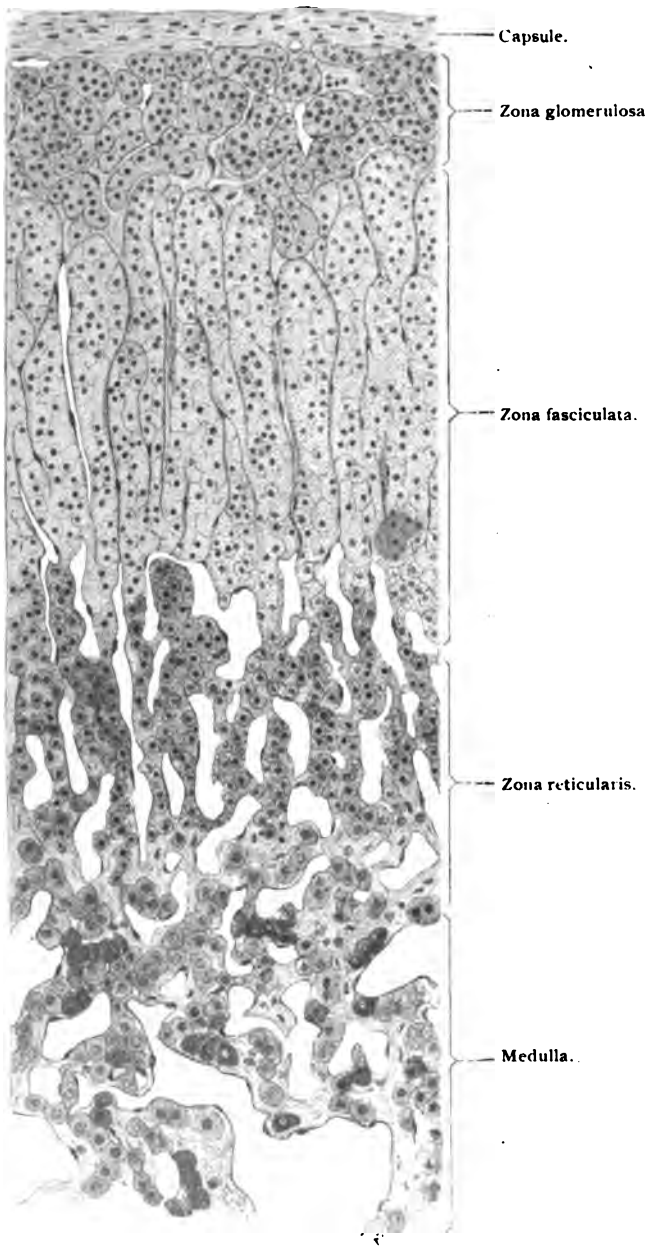



FIG. 131.—SECTION THROUGH CORTEX AND MEDULLA OF THE SUPRARENAL BODY OF ADULT MAN × 200. (Schaper.)

## TECHNIC.

No. 67.—*The Spinal Cord.*—For the study of the distribution of the white and the gray substance the spinal cord of a child should be fixed in toto in about one liter of Müller's fluid, that should be frequently changed; after four or five months thick cross-sections of the cervical, thoracic, and lumbar regions may be cut, and without further treatment mounted in dilute glycerol (p. 22), or after the customary preliminary treatment they may be mounted in damar.

No. 68.—*The Spinal Cord; Staining of Medullated Fibers, after Pal.*—The success of the preparation depends especially on the state of preservation of the organ. The fresher the tissue when it is put into the fixing fluid, the better will be the result. The entire spinal cord should be placed in a large quantity of Müller's fluid, that must be changed daily during the first week and frequently thereafter. If it is desired to investigate only portions of the spinal cord, then place pieces of the fresh cord about 2 cm. long, taken from the lower cervical, the middle thoracic, and the lumbar region, in 200 to 500 c.c. of Müller's fluid or, better, suspend them in it. In four or six weeks, during which time the fluid must be frequently changed, the tissue is to be transferred directly, without previous washing, to 150 c.c. of 70 per cent. alcohol and on the following day to the same quantity of 90 per cent. alcohol. The bottle containing the tissue must be placed in the dark (p. 33), and the alcohol be frequently changed during the first eight days. Sections may then be cut. The sections are to be placed in a capsule containing 20 c.c. of 70 per cent. alcohol, and as soon as possible transferred from this to 30 c.c. of Weigert's hematoxylin to which 1 c.c. of lithium carbonate solution has been added (p. 24). In five or six hours the now very dark, untransparent sections should be transferred to 50 c.c. of distilled water plus 1 c.c. of lithium carbonate solution. In a half-hour, during which time the fluid must be changed several times, the sections will give off no more color and are then to be placed in 30 c.c. of potassium permanganate solution for differentiation (p. 24). In from one-half to three minutes the sections are to be washed for one minute in distilled water and then transferred to 20 c.c. of the acid mixture (p. 24). The capsule containing the acid mixture should be covered. The decolorization occurs in from ten to fifty seconds; the gray substance becomes light yellow, almost white, the white substance (the medullated nerve-fibers) appears very dark. Now transfer the sections to a capsule containing 30 c.c. of distilled water and in five minutes to a second capsule containing the same quantity of fresh distilled water. After ten minutes place them in 10 c.c. of alum-carmine, in which they may remain from three to fifteen hours. Mount in damar. The alum-carmine staining may be omitted.

The foregoing directions are intended for thin, well-fixed preparations. If the sections are thick, if the tissue has lain a long time in alcohol, more time will be required for staining and reduction. Should

they not stain, place unstained sections in Müller's fluid for twenty-four hours, wash one minute in distilled water, then stain, and the result may be successful. Should the decolorization be insufficient, if the gray substance does not become yellowish-white, the procedure may be repeated; that is, the sections are to be again placed in distilled water one minute, then in potassium permanganate one or three minutes, then in distilled water one minute, and finally in the acid mixture. The given quantities of the permanganate solution and of the acid mixture are sufficient for only a few, about 20 sections. If it is desired to treat more sections, larger quantities of these fluids must be used.

No. 69.—*The Spinal Cord; Staining of Axis-cylinders and of Cells.*—Place pieces at the most 2 cm. long in 200 c.c. of Müller's fluid, that must be changed daily during the first week and once a week thereafter. In four weeks transfer the tissue directly from Müller's fluid to about 50 c.c. of sodium carminate (1 per cent. aqueous solution), in which it should remain for three days. *During this time the bottle containing the tissue must be frequently shaken.* The stained pieces are to be washed for twenty-four hours in running water, then placed in 150 c.c. of 70 per cent. alcohol, and after five hours transferred to the same quantity of 95 per cent. alcohol. Mount the cross-sections in damar (Fig. 100).

No. 70.—*Spinal Cord; Golgi Staining.**—The length of time the tissue must remain in the Golgi mixture depends upon the elements it is desired to stain,† as follows:—

Two to three days for neuroglia-cells.

Three to five days for nerve-cells.

Five to seven days for nerve-fibers (collaterals).

For this purpose take the spinal cord with the vertebral column of a newborn rat or mouse, and treat it according to the method given on page 41. Since the pieces must be used as soon as they are taken out of the silver solution, only one piece at a time should be transferred to the absolute alcohol. Cut the sections through the spinal cord and the vertebral column.

The spinal cord of a three- or seven-day-old embryo chick furnishes still better results, but it is necessary to embed the tissue in celloidin (see Microtome Technic). The spinal cord of kittens as well as that of human embryos 20 to 40 cm. long yields very useful results.

No. 71.—*The Brain; Staining of Medullated Nerve-fibers.*—Apply the method given in No. 68. If an entire human brain is to be placed

* *Editor's remark:* The application of the *Cox-Golgi mixture*, in the manner described on p. 41, footnote, is also highly recommended. Since it can be applied with good results to the central nervous system of *adult* animals, it offers, in the manipulation of the material and the preparation of the sections, valuable advantages, particularly to the beginner. After the treatment with alcohol the larger pieces, without being embedded, can be easily cut freehand, when *thick* sections are desired.

† If the action of the mixture is too brief the central portions of the sections appear untransparent and penetrated by abundant precipitates; if the action of the mixture is too prolonged the resulting impregnation of the elements will be unsatisfactory.

in Müller's fluid, many deep incisions should be made in it and about 3 liters of the fixing fluid should be used.

No. 72.—*The Brain; Cells.*—Treat pieces 1 or 2 cm. square of the cerebral cortex (paracentral convolution) and of the cerebellar cortex like No. 69. In the cerebral cortex, in addition to the cell-forms described, an extremely variable number of vesicular spaces containing remnants of cells (protoplasm and nucleus) may be seen (Fig. 132, *z*); they are probably pericellular lymph-spaces, which by post-mortem alteration and the influence of the fixation medium have become abnormally enlarged. The sections through the cerebellar cortex must be made transverse to the long axis of the convolutions, since the ramifications of the cells of Purkinje extend only in planes transverse to the convolution. Only a few cells of Purkinje lie in the depressions between the convolutions.

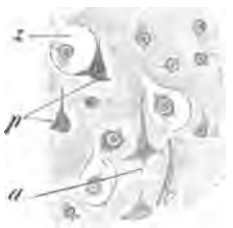



FIG. 132.—PORTION OF A SECTION OF HUMAN CEREBRAL CORTEX. × 240. *p*, Small pyramidal cells; *a*, the nerve-process of a pyramidal cell.

No. 73.—*The Brain; Golgi Staining.**—*a.* For a topographical view, treat the brain of a newborn rat or mouse in the unopened cranium according to the method given in No. 70. The cranium may be sectioned with the brain-substance.

*b.* For specimens of the cortex, the brain of a mouse from eight to thirty days old is most suitable, treated with the Golgi mixture for from two to three days, or of a one- to fifteen-day-old rabbit or a kitten under six weeks old, treated with the Golgi mixture for five days. Pieces of the brain of adults must remain in the Golgi mixture for from eight to fifteen days. Further treatment like No. 70.

No. 74.—*The Cortex of the Cerebellum; Golgi Staining.**—Remove the cerebellum from the cranium of a newborn guinea-pig (or a kitten less than six weeks old) and treat it according to the method given in No. 70. The staining of the elements of the cerebellum is more difficult to accomplish than of those of the cerebrum and the spinal cord. Failures are frequent. The sections should be principally made vertically to the axis of the convolutions. (For embedding, see Microtome Technic.)

No. 75.—*Hypophysis Cerebri.*—Treat like No. 80.

No. 76.—*Brain-sand, Acervulus Cerebri.*—Tease the epiphysis in a drop of salt solution. If much brain-sand is present, a gritty sound will be heard on teasing and the larger concretions can be perceived by the unaided eye. Examine with the low power, without a cover-glass (Fig. 114); the granules are not always round, but often oval and dentated; occasionally the irregularity of the surface is indistinct, because they are

* For the application of the Cox-Golgi mixture see p. 41 and 200, remark.

enveloped in concentrically-arranged connective-tissue fibers. Push aside the larger granules with a needle, cover a few of the smaller ones with a cover-glass and treat with 2 to 3 drops of hydrochloric acid (p. 48). Bubbles of gas develop and the sharp outlines of the granules disappear.

No. 77.—*Corpuscula Amylacea.*—Select the brains of elderly individuals. With a scalpel scrape the mesial surface—that directed toward the third ventricle—of the optic thalamus and spread the scrapings with a needle in a drop of salt solution; apply a cover-glass. The corpuscles when present are easily found, and are recognized by their bluish-green color and their stratification (Fig. 115, *a*). They must not be confused with drops of extruded myelin (*b*), which are always clear and have a double contour. In addition there are found in such preparations numerous red blood-corpuscles, ependymal cells (*d*), medullated nerve-fibers varying in thickness, and ganglion-cells; the latter are very pale and often can only be detected by their pigmentation (*f*). Human brains, even though not absolutely fresh, are still useful.

No. 78.—*a.* Spread out a piece 1 cm. long of the *choroid plexus* in a drop of salt solution and apply a cover-glass. The convoluted red blood-vessels and the epithelium of the plexus can be seen.

*b.* Very pretty permanent preparations may be obtained as follows: Carefully spread out a little piece of the plexus in salt solution; if good fields are visible with the low power, let the salt solution flow off and add a few drops of Zenker's fluid (p. 21); then apply a cover-glass, at the edge of which place a little more of the Zenker's fluid. After thirty minutes displace this fluid by distilled water, and after another thirty minutes the water by 50 per cent. alcohol to which a few drops of tincture of iodin have been added. In fifteen minutes take off the cover-glass and transfer the now fixed preparation to a watch-glass with fresh 50 per cent. iodin-alcohol, to which, in case it becomes rapidly decolorized, tincture of iodin is to be added. In from fifteen to thirty minutes transfer the object to pure 70 per cent. alcohol, and after about twelve hours stain it with hematoxylin and eosin (p. 37) and mount in damar (p. 45).

No. 79.—*Transverse Sections of Nerve-fiber Bundles.*—Treat a piece of nerve, if possible the sciatic of man, that possesses a well-developed endoneurium, according to the method given in No. 32. Place it for six days in a 0.1 per cent. solution of chromic acid, then wash it for from three to four hours in running water, and harden it in gradually-strengthened alcohol. When the hardening is completed, cut thin sections with a *sharp* razor. It is advisable to embed the tissue in liver; better still, in elder-pith or in the pith of the sunflower. For this purpose, make a hole in the dry elder-pith with a needle, and then carefully insert the nerve. Place the whole for about a half-hour in water; the pith swells and firmly grasps the nerve. Stain the sections in picrocarmine and mount in glycerol. The length of time required for staining varies greatly. The sections must be very carefully handled and pressure

with the cover-glass must be scrupulously avoided, lest the sections of the fibers, which are not discs, but short cylinders, be turned on their sides, and not a fiber in section be seen. If successful, the section will show a somewhat shrunken axis-cylinder, resembling a red nucleus, surrounded by the yellow medulla, which is enclosed by the reddish neurilemma. The cross-section of the nerve-fiber has been compared to a picture of the sun (*Sonnenbildchenfigur*) (Fig. 133).

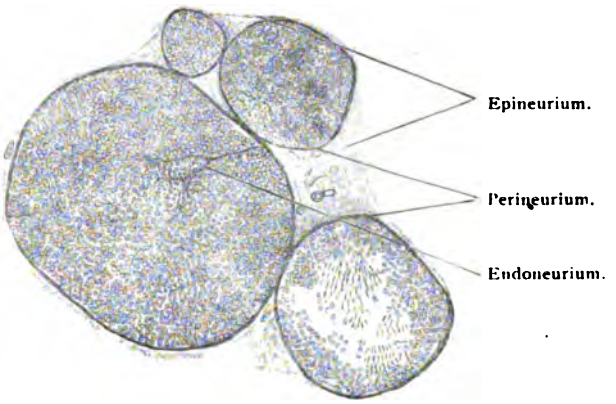



FIG. 133.—FROM A TRANSVERSE SECTION OF A PERIPHERAL (SPINAL) NERVE OF RABBIT. × 50. In the lower funiculus, on the right, some of the transverse sections of nerve-fibers have fallen out, others are lying on one side, as a consequence of pressure. In the rabbit the endoneurium is only slightly developed.

No. 80.—*Spinal Ganglia.*—These are difficult to obtain. Therefore remove the Gasserian ganglion from the depression in which it is lodged (on the anterior surface of the petrous portion of the temporal bone), and place it in about 100 c.c. of Müller's fluid for fixation.* After four weeks wash it for three hours in running water and harden it in 50 c.c. of gradually-strengthened alcohol (p. 33). Cut the thinnest possible transverse and longitudinal sections; stain them thirty seconds in hematoxylin, then from two to five minutes in eosin (p. 37, 3 *b*), and mount in damar. The ganglion-cells are pale red; the axis-cylinder deep red; the medullary sheath brownish; the nuclei blue (Fig. 119 and Fig. 120). If the section is not sufficiently thin, the large number of deeply-stained nuclei will render it difficult to see the other structures. For this reason it is better to stain thick sections in picrocarmine, two or three days, and mount them in damar. The nuclei are then not so intensely stained. Occasionally the protoplasm of the ganglion-cell contracts, and thus acquires a stellate outline (Fig. 120, x), that may easily lead the beginner to confuse it with a multipolar ganglion-cell.

T-shaped branches may be seen in preparations of the spinal cord treated after No. 70. In young embroyo chicks the spinal ganglion-cells are still bipolar. Unipolar cells are found in embryo chicks about

* Fixation in Kleinenberg's picrosulphuric acid also gives very good results.

seventeen days old, transition forms between the ninth and fourteenth days, and in embryo rabbits from 5 to 12 cm. long.

No. 81.—*Sympathetic Ganglia.*—Fix and harden the large superior cervical ganglion of the sympathetic nerve like No. 80. Here, too, on account of the abundance of nuclei, nuclear staining is applicable only to *very* thin sections. Treated according to the method given in No. 80, the processes of the multipolar ganglion-cells are not rendered distinct. For this purpose place the thinnest possible sections for twenty-four hours in 5 c.c. of nigrosin solution (prepared like the methyl-violet solution, p. 25); then transfer them to 5 c.c. of absolute alcohol for five minutes, and preserve in damar. The characteristic bundles of nonmedullated nerve-fibers, cut obliquely and transversely, can be recognized with the low power; also the ganglion-cells; but to see their processes high magnification and careful scrutiny are necessary (Fig. 121). In many sections the processes of the ganglion-cells cannot be seen; the latter may be best exhibited according to the method given in No. 70, and a suitable object is the cervical portion of a ten- or fifteen-day-old embryo chick.

No. 82.—*Simple Tactile-Cells; Intra-epithelial Nerve-fibers; Cells of Langerhans; Tactile Corpuscles.*—Prepare a mixture of gold chlorid and formic acid (p. 43), boil it and let it cool; then cut from the volar side of a freshly-amputated finger or toe (with scissors applied flatwise) several small pieces of the epidermis and uppermost layers of the corium about 5 mm. long and 1 mm. broad. Carefully remove any fat attached to the under surface of the corium and place the pieces in the gold and formic acid mixture for one hour, *in the dark.* Then, with glass-rods, transfer the pieces to 10 c.c. of distilled water and in a few minutes to fresh distilled water to which formic acid has been added (p. 43), and expose the whole to daylight (sunlight is unnecessary). In from twenty-four to forty-eight hours the tissue becomes dark violet. It is now to be hardened in 30 c.c. of gradually-strengthened alcohol. In eight days the pieces may be embedded in liver and sectioned; mount in damar. The epidermis is red-violet in different tints; the nuclei are only to be seen in places and often are not perceptible; the corium is white; the capillaries, the excretory ducts of the coil-glands, and the nerves are dark violet to black. For tactile cells the thinnest possible sections are necessary. They may often be found near the excretory ducts of the coil-glands. Care must be taken not to confuse them with shrunken epithelial-cells (Fig. 123).

The *intra-epithelial nerve-fibers* appear as delicate filaments; their connection with the nerve-fibers in the corium is difficult to trace. Processes of the cells of Langerhans, in thin sections, are apt to be confused with the intra-epithelial nerve-fibers (Fig. 122).

The *cells of Langerhans* and the *tactile corpuscles* may be easily seen; in thick sections the tactile corpuscles are black (Fig. 122), in thin sections red-violet (Fig. 127).

No. 83.—*Compound Tactile Cells.*—Cut the yellowish wax-like skin,

or cere, from the lateral edges of the upper beak of a duck or goose and treat pieces 1 or 2 mm. thick and 1 cm. long with 3 c.c. of 2 per cent. osmic-acid solution plus 3 c.c. of distilled water; place the whole in the dark from eighteen to twenty-four hours; then wash the pieces for one hour in running water and transfer them to 20 c.c. of 90 per cent. alcohol. In six hours the objects may be sectioned. Embed them in liver and make the sections from the corium toward the epithelium, not the reverse. The sections may be mounted unstained in damar. The olive-green tactile cells can be readily seen, but the entrance of the nerve-fiber is difficult to find (Fig. 124). In addition, Herbst's corpuscles occur in the sections. If it is desired to stain the sections, use a nuclear staining solution (p. 36).

No. 84.—*Cylindrical End-bulbs.*—With scissors and forceps cut out pieces 1 cm. square of the scleral conjunctiva near the corneal margin of the fresh eye of a calf, taking care not to roll them. It is better to let them lie smooth on the sclera. Carefully slip the pieces, epithelial side up, from the sclera on to a cork-plate and span them out with needles. Moisten the surface with a few drops of the vitreous humor obtained from the eye of the calf; with scissors and forceps dissect off a thin layer consisting of connective tissue and the epithelium resting upon it. This operation must be done with great care; folding and torsion of the membrane must if possible be avoided. The pieces, with the epithelial side up, should now be slipped on to a dry slide and spread out flat. At first they draw together, but in a moment or two the edges dry somewhat and adhere to the glass and they can then be extended without much difficulty. The slide with the preparation is next to be placed in a glass jar containing 65 c.c. of distilled water to which 2 c.c. of acetic acid have been added. In about an hour (or later), during which time the pieces swell considerably and float from the slide, with a clean needle endeavor to remove the epithelium; it may be loosened without much trouble and floats off in fine white shreds. If this is not done cautiously the end-bulbs lying close beneath the epithelium may be torn off with it. The more thoroughly the epithelium is removed the better. After the pieces have lain four or five hours in dilute acetic acid transfer them with a few drops of the same fluid to a slide, apply a cover-glass and make slight pressure upon it with the outspread branches of the forceps. On examination with the low power the blood-vessels may be distinctly seen—they are recognized by their prominent nuclei—and also the medullated nerve-fibers.* Trace such a fiber until the medulla ceases; examine such places with the high power, for there the end-bulbs are most apt to be found. In many cases nothing will be seen but the numerous nuclei and even when a favorable situation is found the end-bulbs are so pale that it is very difficult to perceive them; the axis-cylinder, too, is often very difficult to see (Fig. 125). Only the prac-

*In the calf some of the nerve-fibers are nonmedullated; these are not recommended for the investigation.

tised microscopist will have much success in finding them. Beginners are advised not to attempt this preparation.

No. 85.—*Lamellar Corpuscles.*—These are best obtained from the mesentery of a cat, where they may be seen with the unaided eye. They appear as milky, glass-like, transparent, oval spots between the strands of adipose tissue of the mesentery. Their number varies greatly. Occasionally they are very scarce and of such small size that to find them requires close searching. Cut out the portion of the mesentery containing the corpuscles, and spread them out in a drop of salt solution on a slide lying on a black background. Endeavor to remove the attached clusters of fat-cells, taking care not to prick the corpuscles. Ascertain with a low power, without a cover-glass, whether the corpuscles have been sufficiently isolated. Cover them with another drop of salt solution and a cover-glass. Pressure must be carefully avoided. The corpuscle represented in Fig. 126 was of very small size.

With the high power one can distinctly see the nuclei of the cells lining the capsules; the oval nuclei of the inner bulb are often indistinct and pale. If it is desired to preserve the preparation, treat it under the cover-glass with 1 or 2 drops of 1 per cent. osmic acid and, after the medulla is blackened and the inner bulb has become brown, displace the acid with very dilute glycerol. Methylene-blue staining (p. 39) is recommended.

No. 86.—*Motor Nerve-endings.—a. Terminal Ramifications.*—Prepare a mixture of 24 c.c. of 1 per cent. gold chlorid solution plus 6 c.c. of formic acid, boil it and let it cool; cut out small pieces 3 or 4 cm. long of the intercostal muscles of a rabbit and treat them like No. 82; after the dark-violet pieces have lain from three to six days in 70 per cent. alcohol, tease a muscle-bundle about 5 mm. broad in a drop of dilute glycerol to which a very small drop of formic acid has been added. It is of advantage to make slight pressure on the cover-glass. To find the terminal ramifications, trace with the low power the easily-recognized black nerve-fibers (Fig. 128). The addition of another drop of acetic or formic acid often renders the elements more distinct.

*b. Nuclei of the Motor Plates.*—Place the anterior halves of the eye-muscles of a recently-killed rabbit in 97 c.c. of distilled water plus 3 c.c. of acetic acid. After six hours transfer the muscles to distilled water; with the scissors cut a thin flat piece and spread it out on a slide; the ramifications of the whitish nerves can be plainly seen with the unaided eye. With low magnification (50 diameters), the anastomoses of the nerve-bundles, as well as the blood-vessels, that are easily recognized by the transversely-placed nuclei of their smooth muscle-fibers, can be seen. On account of the large number of sharply-contoured nuclei belonging to the muscles and the intramuscular connective tissue, the end-plates are not easy to find. If a nerve-fiber be traced it will soon be seen that the double-contoured medullary sheath ceases abruptly and loses itself in a group of nuclei; these are the nuclei of the motor plate, the other

details of which are not distinctly visible. The cross-striation of the muscle-fibers, which are very pale, is often indistinct (Fig. 129).

No. 87.—*The Suprarenal Bodies; Topographical View.*—Fix the entire suprarenal body of a child in 200 c.c. of 0.1 per cent. chromic acid, and after eight days harden it in 150 c.c. of gradually-strengthened alcohol; mount unstained sections in dilute glycerol (Fig. 130, A).

No. 88.—*Elements of the Suprarenal Body.*—Tease portions of the fresh organ in a drop of salt solution. The elements are very delicate and therefore injured cells are of frequent occurrence.

No. 89.—*For the study of the minute structure of the suprarenal bodies*, place 2 cm. cubes of the fresh organ in 100 c.c. of Kleinenberg's picrosulphuric acid and after from twelve to twenty-four hours in an equal quantity of gradually-strengthened alcohol; cut fine sections, stain them in Hansen's hematoxylin, and mount in damar (Fig. 130, B, and Fig. 131). For the exhibition of the nerves, treatment with the Golgi mixture for from six to eight days and with the 0.75 per cent. silver solution for from two to three days, or several repetitions of this procedure, is recommended.

# V. THE DIGESTIVE ORGANS.

## MUCOUS MEMBRANES.

The inner surface of the alimentary tract, of the respiratory organs, of certain parts of the genito-urinary system and of some of the organs of special sense are covered by a soft, moist membrane, the *mucous membrane* or *tunica mucosa*. It is composed of a soft epithelium and of connective-tissue. The latter, immediately under the epithelium, is usually specialized and condensed to form a structureless membrane, the *membrana propria* or *basement membrane*; beneath this follows the tunica propria, which passes by a gradual transition into the subjacent, loose-textured *tunica submucosa*, that in turn connects the mucous membrane with the underlying structures, for example, the muscles or bones. The epithelium of the glands is derived directly from the epithelial elements of the mucous membrane (see p. 71).

### The Mucous Membrane of the Oral Cavity.

The mucous membrane of the mouth consists of three parts: (1) the epithelium, (2) the tunica propria, and (3) the submucosa. The *epithelium* is typical stratified squamous epithelium. The *tunica propria* is formed of interlacing connective-tissue bundles richly interspersed with elastic fibers. The bundles of the uppermost strata are very slender and form a compact, apparently almost homogeneous felt-work. The surface of the tunica propria is beset with numerous usually simple papillæ (Fig. 134, 1), the height of which varies greatly in the separate regions of the oral cavity. The highest papillæ (0.5 mm.) occur at the edge of the lips and on the gums. The tunica propria passes without sharp limits into the *submucosa*, which consists of somewhat thicker bundles of connective tissue, among which the elastic fibers are not numerous. The submucosa is in general loosely attached to the walls of the oral cavity; only on the gums and on the hard palate is it firmer and here intimately united to the periosteum. It contains the glands of the mucous membrane; these are, with the exception of the sebaceous glands occasionally found at the edges of the lips, branched tubular mucous glands from 1 to 5 mm. in size. The main excretory duct (Fig. 134, 2) is somewhat expanded at its lower end and in the greater part of its length

is lined with stratified scaly epithelium ; the branches and twigs into which it divides and subdivides are lined with stratified and simple columnar epithelium respectively. Not infrequently the main excretory duct receives the excretory tubes of small accessory mucous glands (Fig. 134, 2, 3). The minute structure of the gland-tubules will be described with the mucous glands of the tongue. The numerous *blood-vessels* of the oral mucous membrane are arranged in two networks, situated in two horizontal planes, of which the coarser lies in the submucosa,

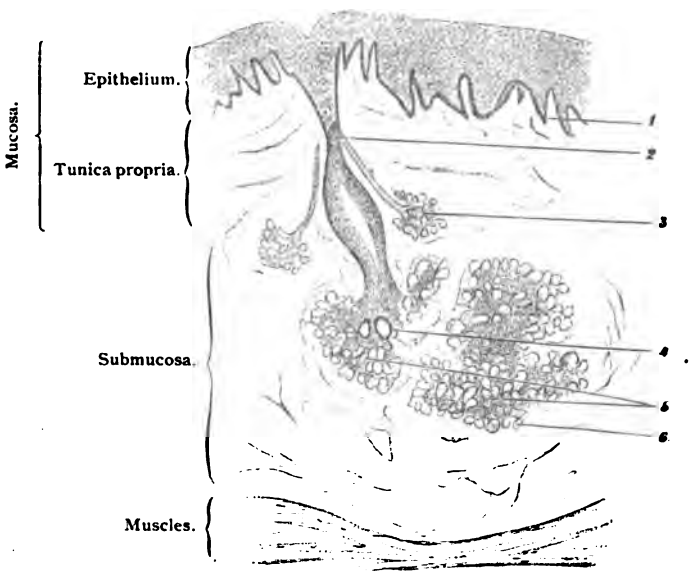



FIG. 134.—VERTICAL SECTION THROUGH THE MUCOUS MEMBRANE OF THE LIP OF ADULT MAN. × 30. 1, Papilla ; 2, excretory duct ; the lumen is cut at only one point ; 3, accessory gland ; 4, a branch of the excretory duct in transverse section ; 5, gland-follicles grouped into lobules by connective tissue ; 6, a gland-tubule in transverse section. Techn. No. 91.

the other finer in the tunica propria. From the latter terminal capillary loops ascend into the papillæ. The *lymph-vessels* similarly form two networks, a coarser in the submucosa, a finer in the tunica propria. The medullated *nerve-fibers* form a wide-meshed reticulum in the submucosa, from which many ramifying fibers ascend to the tunica propria. Here they terminate either in end-bulbs or lose their medullary sheath and as non-medullated nerve-fibers penetrate into the epithelium, where after repeated division they terminate in free endings.

## THE TEETH.

The teeth of man and the higher animals are solid structures, which enclose in their interior a cavity, the *pulp-cavity*, filled with a soft mass,

the *dentinal pulp*. The portion of the tooth within the alveolus or socket is called the *fang*, the exposed portion the *crown*; the juncture of these portions forms the *neck*; the latter is covered by the gums. The hard substance of the tooth consists of three different parts: (1) the *dentine*, (2) the *enamel* with the *enamel cuticle*, and (3) the *cementum*. The disposition of these parts is as follows: the dentine, which contributes the chief bulk of the tooth and determines its form, encloses the pulp-

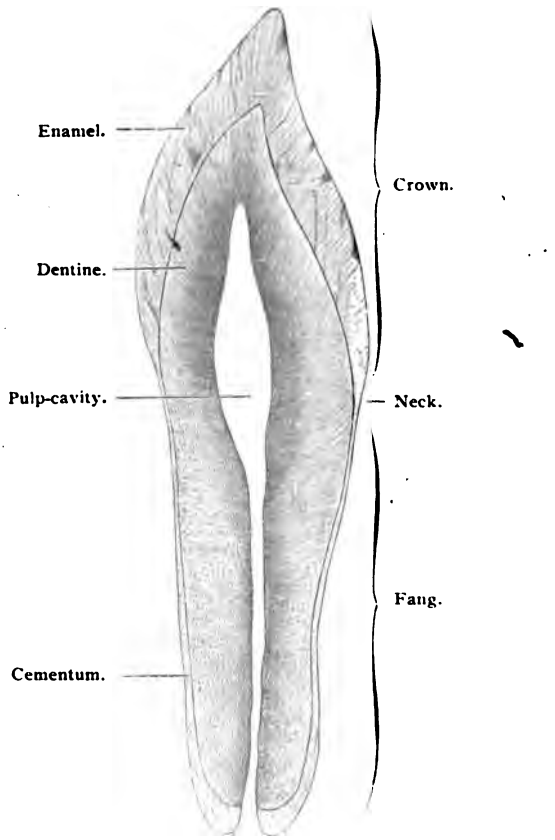



FIG. 135.—LONGITUDINAL SECTION OF HUMAN TOOTH. × 4. Techn. No. 92.

cavity except at the apex of the fang where a narrow nutrient canal admits the nerves and blood-vessels to the pulp; the dentine of the crown is covered by the enamel, of the fang by the cementum, so that its surface is nowhere exposed (Fig. 135).

The *dentine* or *ivory* (substantia eburnea) is a white, opaque mass, harder than bone. It consists of an apparently homogeneous ground-substance containing very fine fibrillæ, which is pierced by numerous

minute channels, the *dentinal tubules*. The latter begin with a diameter of about 2.5 $\mu$ at the inner surface of the dentine, describe an S-shaped curve and then, steadily decreasing in caliber, proceed in a slightly wavy course, radially directed toward the outer surface of the dentine; there they either terminate at the juncture of the dentine and enamel in tapering ends or they form a loop and turn into a neighboring tubule. During their entire course they send off numerous lateral branches, which establish communication with neighboring canaliculi. The matrix immediately surrounding the dentinal tubules is especially dense and forms the so-called *dentinal sheaths*. The lumen of the *dentinal tubules* is occupied by the *dentinal fibers*. At the peripheral parts of the dentine are the *interglobular spaces*, irregular spaces varying in size and filled

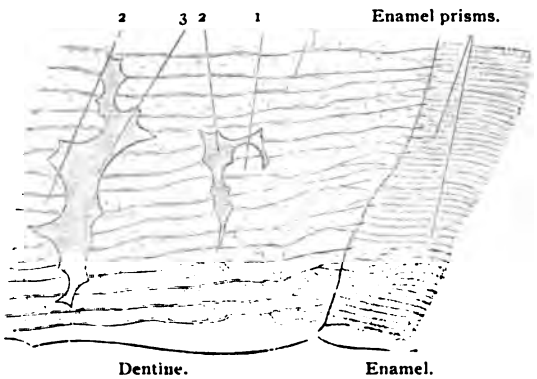



FIG. 136.—FROM A LONGITUDINAL SECTION OF THE LATERAL PART OF THE CROWN OF A HUMAN MOLAR TOOTH. × 240. 1, Dentinal tubules, extending for a short distance into the enamel; 2, dentinal globules projecting into, 3, the interglobular spaces. Techn. No. 92.

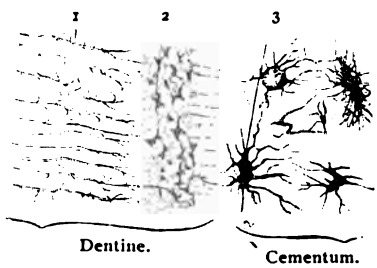



FIG. 137.—FROM A LONGITUDINAL SECTION OF THE FANG OF A HUMAN MOLAR TOOTH. × 240. 1, Dentinal tubules interrupted by a granular stratum, with many, 2, small interglobular spaces; 3, bone-corpuscles with many processes. Techn. No. 92.

with a soft mass; into these spaces the dentine juts in the form of usually hemispherical protuberances, the *dentinal globules* (Fig. 136 and 137). At the neck and in the fang are many very small interglobular spaces; they form the so-called granule stratum lying immediately beneath the cementum.

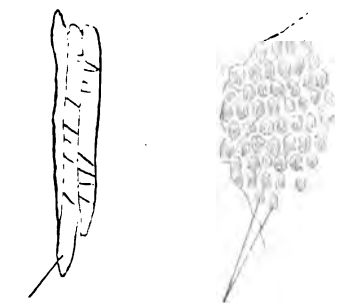
The *enamel* (substantia adamantina) is still harder than the dentine. It is exclusively composed of long, hexagonal, homogeneous fibers,* from 3 to 6 $\mu$ in thickness, the *enamel prisms* (Fig. 138), which are firmly united with one another by a scanty amount of irriguous cement-substance. They extend radially, with many undulations, from the sur-

* The transverse bands do not appear until after treatment with reagents.

face of the dentine to the free surface of the enamel; this is covered by a very thin but very resistant membrane, the *enamel cuticle.*

The *cementum* (substantia ossea) coincides in its structure with that of bone. It contains many Sharpey's fibers. Haversian canals are found only in the cementum of older individuals; stratification in lamellæ is seldom well defined. Bone-corpuscles are absent near the neck.

The space between the fang and the alveolus is occupied by the richly-innervated periosteum, which is firmly united to the cementum by Sharpey's fibers, which penetrate from the inferior maxilla through



Enamel prisms, isolated. Enamel prisms in transverse section.

Fig. 138.—Enamel Prisms from the Tooth of an Infant. Techn. No. 94.

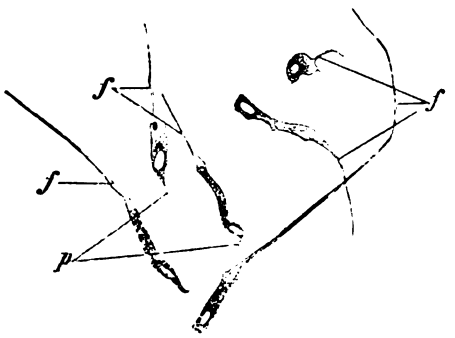



Fig. 139.—Six Odontoblasts with Dentinal Fibers, *f*; *p*, pulp processes. From the pulp of an infant. × 240. Techn. No. 93.

the periosteum into the cementum. The uppermost portion of the periosteum is called the *circular dentinal ligament.*

The *dentinal pulp* is formed of a soft connective tissue containing delicate fibers not united in bundles, the cellular elements of which, on the surface toward the dentine, form a layer of elongated nucleated cells, the *odontoblasts;* these send out short processes, pulp processes (Fig. 139), that are connected with other elements in the pulp, and long processes that extend into the dentinal tubules as the above-mentioned *dentinal fibers* (Fig. 139, *f*). Blood-vessels and nerves are limited to the pulp of the tooth.

## Development of the Teeth.

The development of the teeth in man begins toward the close of the second month of fetal life * and is first indicated by a linear proliferation of the primitive epithelium of the oral cavity, which in the form of a continuous projection grows obliquely into the subjacent connective tissue.

* That which, at an earlier period (fortieth day), has been described as the anlage, is not this alone, but includes the anlage of the labial furrow.

This projection, the *dental ridge* ("enamel germ") (Fig. 140, A) develops on its lateral (labial) surface knob-like protuberances, the *dental*

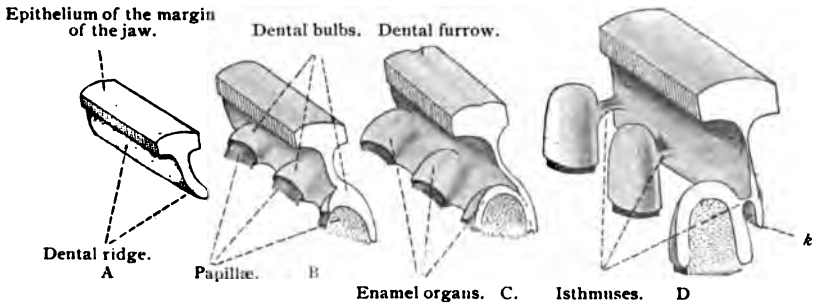



FIG. 140.—SCHEMATIC REPRESENTATION OF THE INITIAL PROCESSES IN THE DEVELOPMENT OF THE TEETH, showing the formation of three teeth. The anlage of each anterior tooth is seen in section; the cut surface is stippled. *k*, Free edge of the dental ridge.

*bulbs* (B), corresponding in number to the temporary teeth, while coincidently in the surrounding mesoderm as many conical aggregations of closely-packed connective-tissue cells arise, the young *dental papillæ*

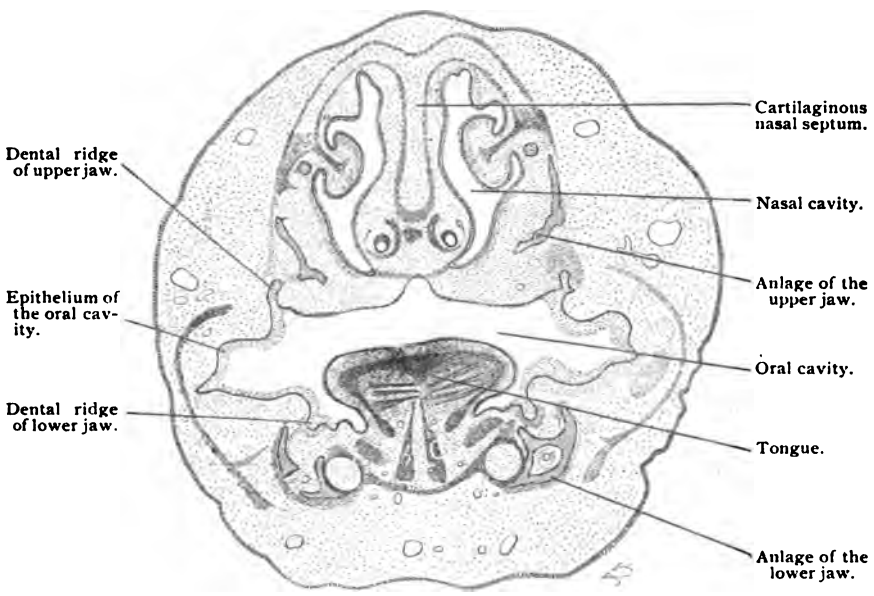



FIG. 141.—FRONTAL SECTION OF THE HEAD OF AN EMBRYO SHEEP, 4 CM. LONG. × 15. Techn. No. 95.

(B) (tenth week). The latter advance obliquely from the labial side out of the depths to the lingual side toward the surface and are embraced by the dental bulbs in such a manner that these form an epithelial cap

for the dental papillæ. Thus each bulb becomes an *enamel organ.* Meanwhile the dental ridge has assumed a more nearly vertical position (C). At about this time, too, a longitudinal groove on the margin of the jaw is visible, the *dental furrow*, which exteriorly marks the region from which the dental ridge grew into the depths. The time of the appearance of the dental furrow varies; frequently it is present in the initial stages. It disappears later. The original broad attachment between the dental ridge and the enamel organ becomes diminished by partial constriction and finally is reduced to a slender cord, the *isthmus* (Fig. 140, D). Meanwhile the papilla and enamel organ grow beyond the dental ridge into the depths, so that the free edge of the latter does

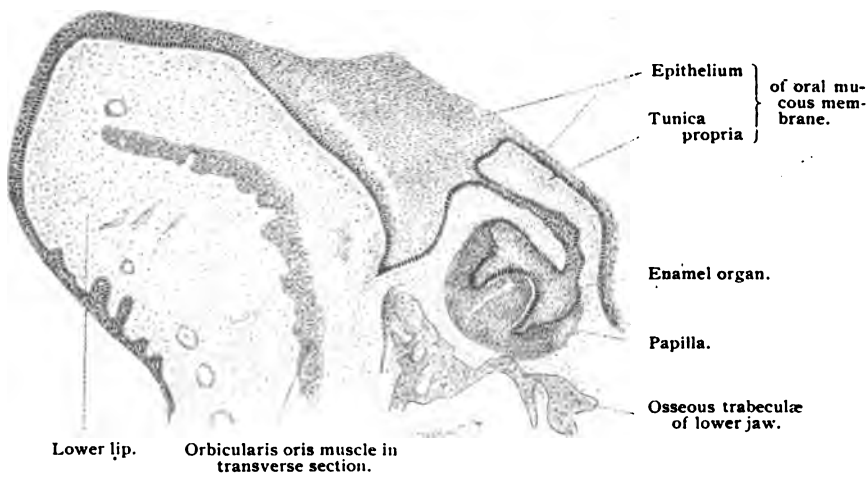



FIG. 142.—CROSS-SECTION OF THE LOWER JAW OF A HUMAN EMBRYO FOUR MONTHS OLD. × 42. Techn. No. 95.

not extend to half the length of the enamel organ (Fig. 140 and Fig. 143).

At the same time the elements of the enamel organ undergo differentiation. The inner layer of cells, resting upon the papilla, develop into tall columnar elements, the *inner enamel cells* (Fig. 143); their inner surface is provided with a cuticular border. The peripheral cells (Fig. 143), steadily decrease in height (Fig. 144), until finally they are reduced to thin plates, the *outer enamel cells;* the cells between the inner and the outer enamel cells, by an abundant increase of the intercellular substance, become transformed into stellate, anastomosing elements, and form the *enamel pulp* (Fig. 143 and Fig. 144). At the point where the inner enamel cells bend over into the layer of outer enamel cells, the enamel organ grows further into the depths until it has reached the lowest ex-

tremity of the anlage of the tooth. Thus the enamel organ, in a measure, forms the matrix in which the tooth develops. The determination of the shape of the future tooth is the first function of the enamel organ; the second is the production of the enamel, which takes place only in that portion of the layer of inner enamel cells enveloping the crown of the tooth. This portion may be named the *enamel membrane*. Each cell of this membrane deposits a substance which subsequently calcifies and

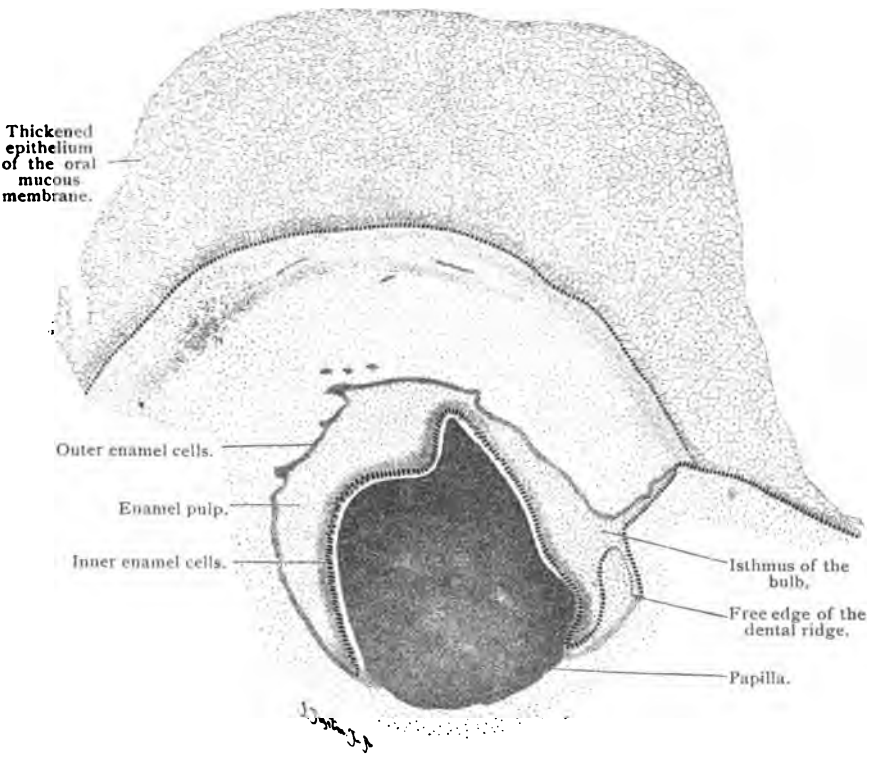



FIG. 143.—FROM A CROSS-SECTION OF THE UPPER JAW OF A HUMAN EMBRYO FIVE MONTHS OLD. × 42. Techn. No. 95.

becomes an enamel prism. The inner enamel cells surrounding the fang take no part in the production of the enamel; they decrease in height and (since here the enamel pulp soon disappears) apply themselves directly against the outer enamel cells, the two layers forming the *epithelial sheath* of the fang (Fig. 144).

Before the production of enamel has begun the first lamina of dentine has been formed (about the twentieth week). The superficial cells of the dental papilla elongate and become the *odontoblasts*, the agents

which produce the at first uncalcified dentine (Fig. 144). The odontoblasts do not develop beyond the extent of the epithelial sheath. As soon as the first dentine is formed, the epithelial sheath undergoes regressive

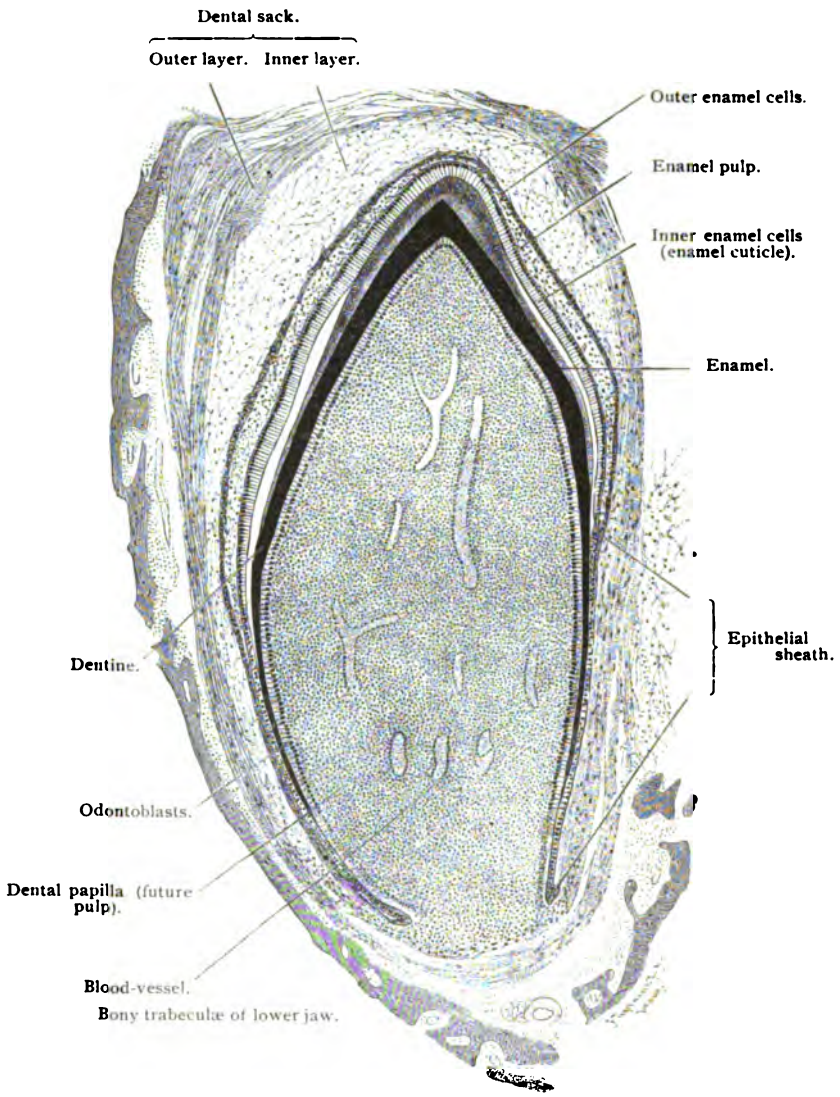



FIG. 144.—LONGITUDINAL SECTION OF A YOUNG MILK-TOOTH OF A NEWBORN DOG. × 42. Techn. No. 95.

change, since connective-tissue ingrowths from the alveolar periosteum penetrate between the epithelial-cells. This regression begins at the lower border of the enamel organ, thus severing the connection between

the latter and the deepest part of the epithelial sheath. With the completed growth of the tooth the last remnant of the epithelial sheath disappears.

Before the production of enamel and dentine the connection between the dental ridge and the surface is severed * (Fig. 140, D); the mesodermic tissue surrounding the anlage of the tooth forms a compact membrane, the *dental sack*, in which subsequently an inner looser and an outer denser stratum can be distinguished (Fig. 144). The enamel cuticle and the cementum do not appear until after birth, shortly before the irruption of the tooth. The former is produced by the merging of the cuticular borders of the enamel cells into a firm, homogeneous membrane; the latter is a product of the alveolar periosteum.

The permanent teeth develop in the same manner as the temporary teeth; in the twenty-fourth week new protuberances, dental bulbs, develop on the edge of the dental ridge growing further into the depths, that embrace new papillæ penetrating from the side. The anlage of the permanent tooth at first lies in the same alveolus with the temporary tooth; a separate alveolus is developed later. The completed tooth is in part of epithelial origin (the enamel), in part derived from the connective-tissue dental papilla (the dentine), the remains of which persist in the adult as the dentinal pulp. The cementum is in a measure an accessory formation contributed by neighboring tissues.

## THE TONGUE.

The bulk of the tongue is formed of striated muscles, the separate bundles and fibers of which interlace in various directions, that for the greater part of their extent are covered by a reflection of the oral mucous membrane. The bundles of the muscular tissue are disposed in three planes: (1) *vertically* and somewhat *radially* (geniohyoglossus, lingualis, and hyoglossus); (2) *transversely* (lingualis); and (3) *longitudinally* (lingualis and styloglossus). Since the muscle-bundles cross one another for the most part at right angles, sections exhibit a beautiful network. A median septum, the *septum linguæ*, divides the muscular tissue into a right and a left half. The septum begins low at the hyoid bone, gradually increases in height, attains its greatest elevation in the middle of the tongue, then gradually slopes down forward and disappears; it does not extend through the entire half of

* The dental ridge has previously become a perforated plate, beset on all sides with short, jagged excrescences. Remains of the dental ridge may be found in the gums of newborn children and were erroneously regarded as glands (glandulæ tartaricæ).

the tongue, but ceases at a distance of about 3 mm. from the dorsum of the organ. The septum is composed of compact connective tissue.

The mucous membrane of the tongue, like that of the oral cavity, consists of an epithelium, a tunica propria, and a submucosa, but is characterized by the conspicuous development and complicated form of the papillæ. Three kinds of papillæ are distinguished: the *filiform* or *conical*, the *fungiform* or *clavate*, and the *circumvallate papillæ*.

The *filiform papillæ* are cylindrical or conical elevations of the tunica propria, bearing on the summit from five to twenty small secondary papillæ. They are composed of distinctly-fibrillated connective-tissue and numerous elastic fibers, covered by a thick layer of stratified scaly epithelium that over the secondary papillæ not infrequently forms a number of filamentous, horny processes. The filiform papillæ are very

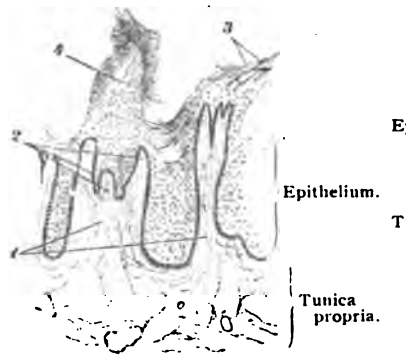



FIG. 145.—LONGITUDINAL SECTION OF THE MUCOUS MEMBRANE OF THE DORSUM OF THE HUMAN TONGUE. × 30. 1, Section of two filiform papillæ, each of which bears, 2, three secondary papillæ; 3, compound, 4, simple process of epithelium, the surface of which is covered with masses of loosely-attached, scaly epithelial-cells. Techn. No. 96.

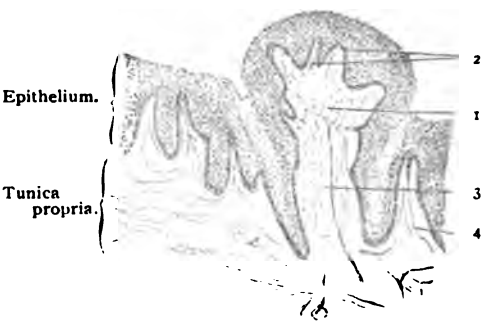



FIG. 146.—LONGITUDINAL SECTION OF THE MUCOUS MEMBRANE OF HUMAN TONGUE. × 30. 1, Fungiform papilla with, 2, secondary papillæ; 3, stalk of fungiform papilla; 4, small filiform papilla. Techn. No. 96.

numerous and are distributed over the entire surface of the tongue; they vary in height from 0.7 to 3 mm. (Fig. 145).

The *fungiform papillæ* are rounded elevations connected with the tunica propria by a slightly-constricted stalk; their entire surface is beset with secondary papillæ. They consist of a distinct feltwork of connective-tissue bundles, which contain but few elastic fibers. The epithelial cover is thinner than that on the filiform papillæ and is not cornified. The fungiform papillæ, not so numerous as the filiform, are also distributed over the entire surface of the tongue and vary in height from 0.5 to 1.5 mm. In the living they are usually easily distinguished by their red color, due to the capillaries shimmering through the transparent epithelium (Fig. 146).

The *circumvallate papillæ* resemble broad, flattened fungiform papillæ and are separated from the surrounding epithelium by a circular

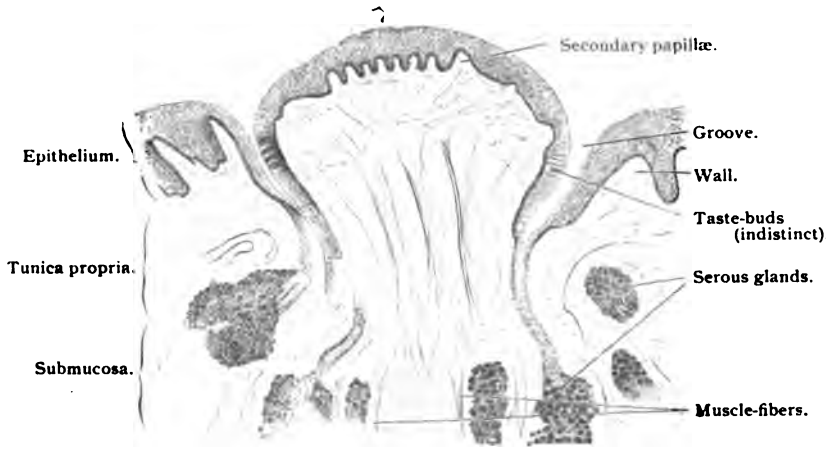



FIG. 147.—VERTICAL SECTION OF A CIRCUMVALLATE PAPILLA OF MAN. × 30. Techn. No. 96.

furrow varying in depth and bounded by a ridge designated the *wall*. The papillæ are composed of connective tissue, like that of the fungiform

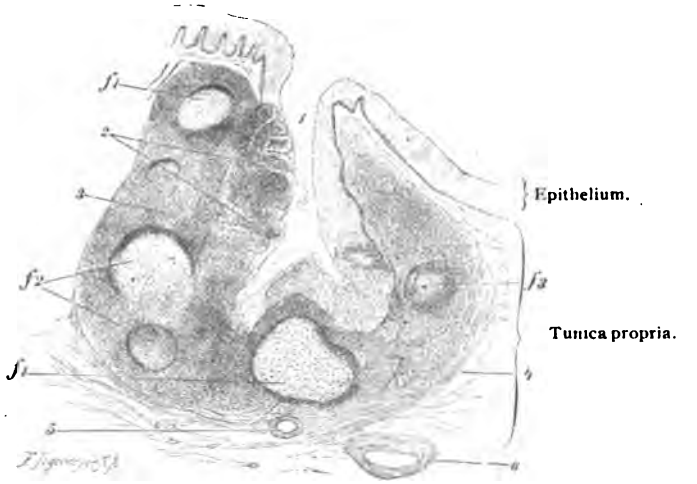



FIG. 148.—VERTICAL SECTION OF A LYMPH-FOLLICLE FROM THE ROOT OF THE TONGUE OF ADULT MAN. × 20. 1. Crypt of the follicle, containing migrated leucocytes. 2. Epithelium of the crypt, infiltrated with leucocytes on the left and at the base, almost intact on the right. 3. Nodules of adenoid tissue containing germinal centers: $f^1$, nodules cut through the middle; $f^2$, through the side; $f^3$, at the periphery. 4. Fibrous sheath. 5. Section of excretory duct of mucous gland. 6. Blood-vessel. Techn. No. 96.

papillæ. Secondary papillæ are found only on the upper, not on the lateral surfaces. In the epithelium covering the sides, and occasionally

also the wall, lie the end-organs of the gustatory nerves, the *taste-buds*. The circumvallate papillæ are few in number, from 8 to 15, and only occur at the posterior end of the dorsum of the tongue. They are from 1 to 1.5 mm. high and 1 to 3 mm. broad (Fig. 147). At the lateral margins of the tongue, near the anterior pillars of the fauces, is a group of parallel folds of the mucous membrane, the papilla foliata, that are

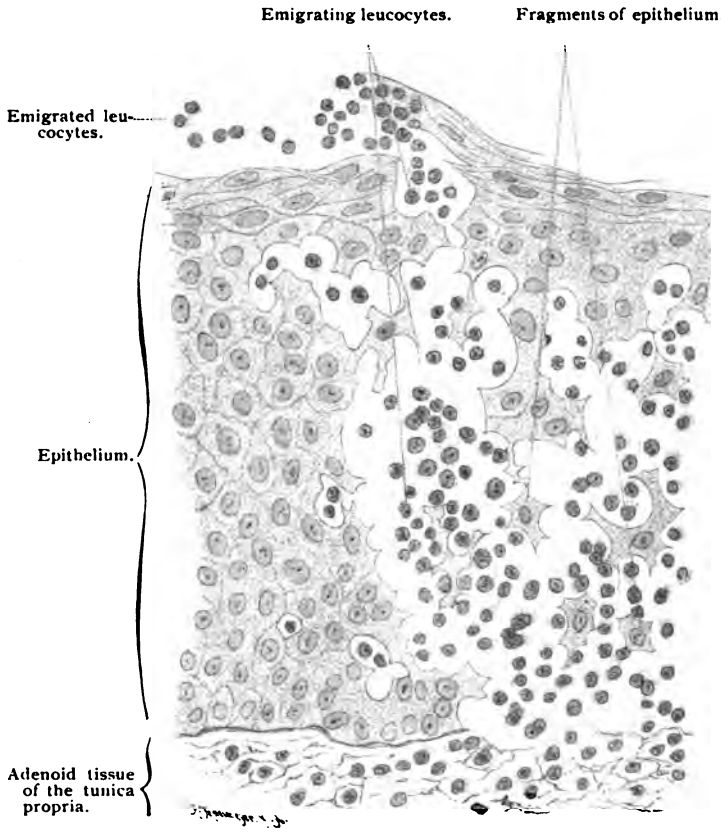



FIG. 149.—FROM A THIN SECTION OF A LINGUAL FOLLICLE OF MAN. × 420. On the left the epithelium is free from leucocytes, on the right many leucocytes are wandering through. In this way the epithelium is undermined and smaller or larger fragments of it are seen lying between the broad passages made by the leucocytes. Techn. No. 96.

distinguished by their wealth of taste-buds. The papillæ foliatæ are especially well developed in the rabbit.

The *submucosa* at the tip and at the edges of the tongue is firm and resistant (fascia linguæ), and intimately connected with the underlying parts.

*The Lymph-follicles of the Tongue* (folliculi linguales).—The mucous membrane of the root of the tongue extending from the circumvallate

papillæ to the epiglottis is peculiarly modified by the development of lymph-nodules. They are spherical aggregations of adenoid tissue from 1 to 4 mm. in size, that embedded in the uppermost strata of the tunica propria form easily-perceptible macroscopic elevations. In the middle a punctate opening may be seen, the entrance to a deep central crypt, lined by a continuation of the stratified epithelium of the oral mucous membrane. Encircling this epithelium is a zone of diffuse adenoid tissue, which contains a variable number of lymph-nodules with germinal centers and is sharply separated from the fibrillar connective tissue of the tunica propria; when the follicles are well developed, the fibrous bundles of the tunica propria are circularly disposed about the adenoid tissue and so form a *fibrous capsule* (Fig. 148, 4). Under normal conditions numerous leucocytes of the adenoid tissue continually wander through the epithelium into the central crypt and from there to the oral cavity; they are readily found in the saliva, as "mucous" and "salivary" corpuscles. The epithelium is often greatly expanded in consequence and destroyed, or is infiltrated with leucocytes to such a degree that its boundary cannot be definitely determined (Fig. 149).

*The Glands.*—Two kinds of branched tubular glands occur in the mucous membrane and in the superficial muscular strata of the tongue. The gland-cells of the one kind produce a mucigenous secretion (mucin); they are named *mucous glands.* The secretion of the second kind is a thin, watery, serous fluid, distinguished by the large amount of albumin it contains; they are called *serous glands.*

FIG. 150.—FROM A SECTION THROUGH THE ROOT OF THE TONGUE OF A MOUSE. × 90. A serous gland; the tubular system silvered by Golgi's "black reaction"; the tubular structure is easily recognized. Techn. No. 119.

The *mucous glands* are of the same structure as those of the oral mucous membrane and occur along the edges and in larger numbers at the root of the tongue, where not infrequently their excretory ducts open into the crypts of the follicles. The ducts are lined by columnar epithelium, which occasionally is ciliated. The walls of the tubules consist of a structureless membrana propria and gland-cells; the latter are columnar elements possessing a firm cell-membrane and vary in appearance with their periodic functional state. The exhausted cell is smaller, the transverse-oval nucleus near the base of the cell (Fig. 151, *I*, *b*); the cell loaded with secretion is broader, the nucleus is pressed flat against the cell-wall (Fig. 151, *I*, *c*, *II*). Generally the same mucous gland, often the same tubule, exhibits different phases of secretion; however, demilunes are not formed here, because the rigid membrane of the

gland-cells resists the pressure exerted by neighboring cells.* The anterior lingual gland (*glandula lingualis anterior*) (Nuhn) occurring in the tip of the tongue also is a mucous gland.

The *serous glands* are limited to the vicinity of the papillæ circumvallatæ and foliatæ; the excretory ducts open into the furrows between the papilla and the wall (Fig. 147), and are lined by simple or stratified, not infrequently ciliated, columnar epithelium. The small tubules consist of a delicate membrana propria and short cylindrical or conical cells, destitute of a membrane, the dim, granular protoplasm of which encloses a round nucleus lying in the middle of the cell (Fig. 151, *IV* and *V*). The lumen of the tubules, especially in animals, is very narrow.

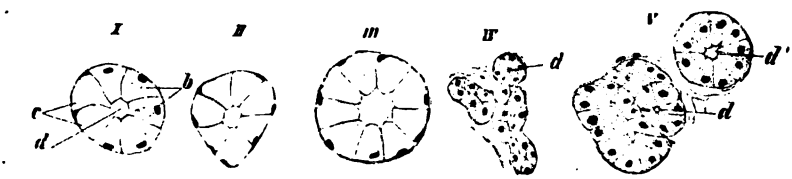



FIG. 151.—*I*, *II*. FROM A SECTION OF A MUCOUS GLAND OF THE ROOT OF A HUMAN TONGUE. *I*, Section of a tubule with (*b*) gland-cells empty of secretion and (*c*) gland-cells filled with secretion; *d*, lumen. *II*. Cross-section of a tubule containing only cells loaded with secretion. *III* and *IV*. From the mucous membrane of the tongue of a rabbit. *III*. Tubule of a mucous gland in transverse section. *IV*. Several tubules of a serous gland, at *d* the very small lumen. *V*. Several tubules of a human serous gland, with large (*d'*) and small (*d*) lumen. All the sections are magnified 240 times. Techn. No. 96.

The *blood-vessels* of the mucous membrane of the tongue form networks disposed parallel to the surface, from which twigs ascend to all the papillæ up into the secondary papillæ. At the root of the tongue small arteries pierce the fibrous envelopes of the lymph-follicles and break up into capillaries that penetrate to the interior of the nodules. The blood-vessels of the glands form capillary networks around the gland-tubules.

The *lymph-vessels* of the tongue are arranged in two sets; a deep set consisting of larger vessels, and a superficial set, which takes up the lymph-vessels of the papillæ. The lymph-vessels at the root of the tongue are very richly developed; they form networks encircling the lymph-nodules.

The *nerves* of the mucous membrane of the tongue, the glossopharyngeal and the lingual branch of the fifth are furnished with small groups of ganglion-cells along their course; their endings behave partly as in other portions of the oral mucous membrane, partly they enter in intimate relation with the taste-buds.

* Only the mucous glands of the tongue of the cat and of the uvula of man exhibit demilunes.

## THE PHARYNX.

The wall of the pharynx is composed of three coats: a *mucous*, a *muscular*, and a *fibrous coat*. The *mucous coat*, like the oral mucous membrane, possesses a stratified scaly epithelium, a tunica propria beset with papillæ, and also numerous mucous glands. The upper or respiratory part of the pharynx (*pars nasalis*) is clothed by stratified ciliated columnar epithelium, the lower limit of which is tolerably variable. Very richly developed is the adenoid tissue. Between the pillars of the fauces it forms conspicuous accumulations, one on either side, known as the palatine tonsils (*tonsilla palatina*), which in respect to their structure in man and many animals correspond to an aggregation of large lymph-nodules like those of the root of the tongue. The leucocytes that wander through the epithelium of the tonsils are so numerous that the latter may be regarded as the most fertile source of the salivary corpuscles. Many mucous glands lie in the neighborhood of the tonsils. The adenoid tissue is also vigorously developed in the respiratory portion of the pharynx, where on the posterior wall between the orifices of the eustachian tubes it forms a conspicuous mass, the "pharyngeal tonsil," which agrees in structure with the palatine tonsils, excepting that the lymphoid tissue is less sharply circumscribed. Here, too, many leucocytes migrate through the epithelium. The development of the adenoid tissue of the oral cavity and of the pharynx is subject to considerable variation.

The *muscular coat* (constrictor muscles of the pharynx) consists of striated muscle-fibers, the description of which belongs to the domain of macroscopic anatomy. The *fibrous tunic* is a stout membrane composed of a dense feltwork of fibro-elastic tissue. Blood-vessels, lymph-vessels, and nerves are distributed in the same manner as in the oral mucous membrane.

## THE ESOPHAGUS.

The wall of the esophagus comprises a mucous, a muscular, and a fibrous coat. The *mucous coat* is composed of a stratified squamous epithelium, of a tunica propria beset with papillæ, following this of a stratum of longitudinally-disposed smooth muscle-fibers, the *muscularis mucosæ;* subjacent to the latter is the *submucosa*, which consists of loosely-joined bundles of connective tissue, which in the upper half of the esophagus contains small mucous glands. The *muscular tunic*, in the upper portion of the tube, is composed of striated muscle-fibers, which in the lower portion are replaced by smooth muscle-fibers. The latter are

arranged in two strata, an inner circular, in which the direction of the muscle-fibers is not everywhere exactly transverse, and an outer longitudinal not continuous layer. The *fibrous coat* consists of compact connective-tissue bundles interspersed with numerous elastic fibers. The distribution of the blood-vessels, lymph-vessels, and nerves is the

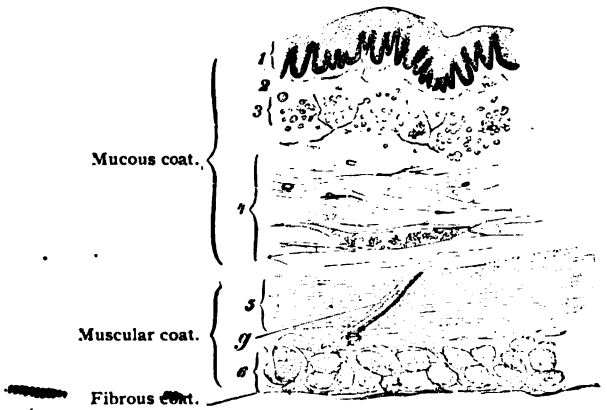



FIG. 152.—FROM A CROSS-SECTION OF THE MIDDLE THIRD OF THE HUMAN ESOPHAGUS. × 10. 1. Stratified squamous epithelium. 2. Tunica propria. 3. Muscularis mucosæ. 4. Submucosa. 5. Circular muscles. 6. Longitudinal muscles. *g*, Blood-vessel. Techn. No. 98.

same as in the pharynx. Between the circular and the longitudinal layers of the muscular coat the nerves form a plexus, at the nodal points of which minute groups of ganglion-cells occur (see plexus myentericus, p. 238).

## THE STOMACH.

The wall of the stomach is from 2 to 3 mm. thick and comprises three coats: a mucous, a muscular, and a serous or fibrous tunic.

The *mucous coat*, sharply contrasted with the white esophageal mucous membrane by its reddish-gray color, consists of an epithelium, a tunica propria, a muscularis mucosæ, and a submucosa (Fig. 153).

The *epithelium* is a simple columnar epithelium, the elements of which produce a mucoid secretion. Two zones can usually be distinguished, an upper mucoid (Fig. 15, *c*), and a lower protoplasmic (Fig. 15, *p*); the latter contains the oval, round, or flattened nucleus. The extent of the mucoid zone varies considerably with the functional phase (cf. Fig. 15). After the discharge of their mucoid contents the epithelial elements closely resemble goblet-cells. The *tunica propria* is composed of a mixture of fibrillar and reticular connective tissue and of an extremely variable number of leucocytes, that occasionally lie closely aggregated

and form solitary lymphatic nodules. The tunica propria contains so many *glands* that its tissue is limited to delicate septa between and to a thin stratum below the tubules. In the pyloric end the glands are farther apart, the tunica propria is conspicuously developed and not infrequently elevated in filamentous or leaf-like villi.

Two kinds of gastric glands are recognized: *fundus glands** (glandulæ gastricæ propriæ), chiefly situated in the middle and cardiac thirds of the stomach, and *pyloric glands*, confined to the narrow pyloric region. Both are simple tubular glands, often branched, especially in the pyloric region, which open singly or in groups into minute, pit-like *depressions*, the *gastric pits* (foveolæ gastricæ), in the mucous membrane of the free surface. The portion of the gland adjoining these depressions is called the *neck*, the following portion the *body*, and the blind end the *fundus* (Fig. 154). Each gland consists of a membrana propria and of gland-cells.

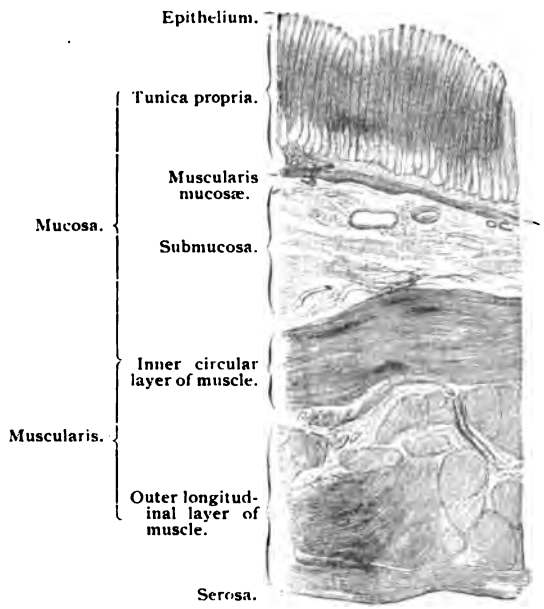



FIG. 153.—TRANSVERSE SECTION OF HUMAN STOMACH. × 15. The tunica propria contains glands standing so close together that its tissue is visible only at the base of the glands toward the muscularis mucosæ. Techn. No. 99.

The *fundus glands* contain two kinds of cells, *chief-* or *central-cells* and *parietal-* or *acid-cells.*† The former are clear, cubical, or short columnar cells, with a granular protoplasm surrounding a spherical nucleus. The chief-cells are very unstable. The parietal-cells are usually considerably larger, darker, and of a rounded or triangular form; their granular

* In the earlier text-books the fundus glands were called peptic glands, a name based upon a function of the glands now called into question.

† The assertion upheld on various sides that the chief- and the parietal-cells are different functional appearances of one kind of cells, as also the statement that during digestion the parietal-cells multiply, but disappear after prolonged fasting, are very much in need of thorough investigation. The stomach of an animal killed after a long winter hibernation still contains parietal-cells.

protoplasm surrounds a spherical nucleus. The parietal-cells are

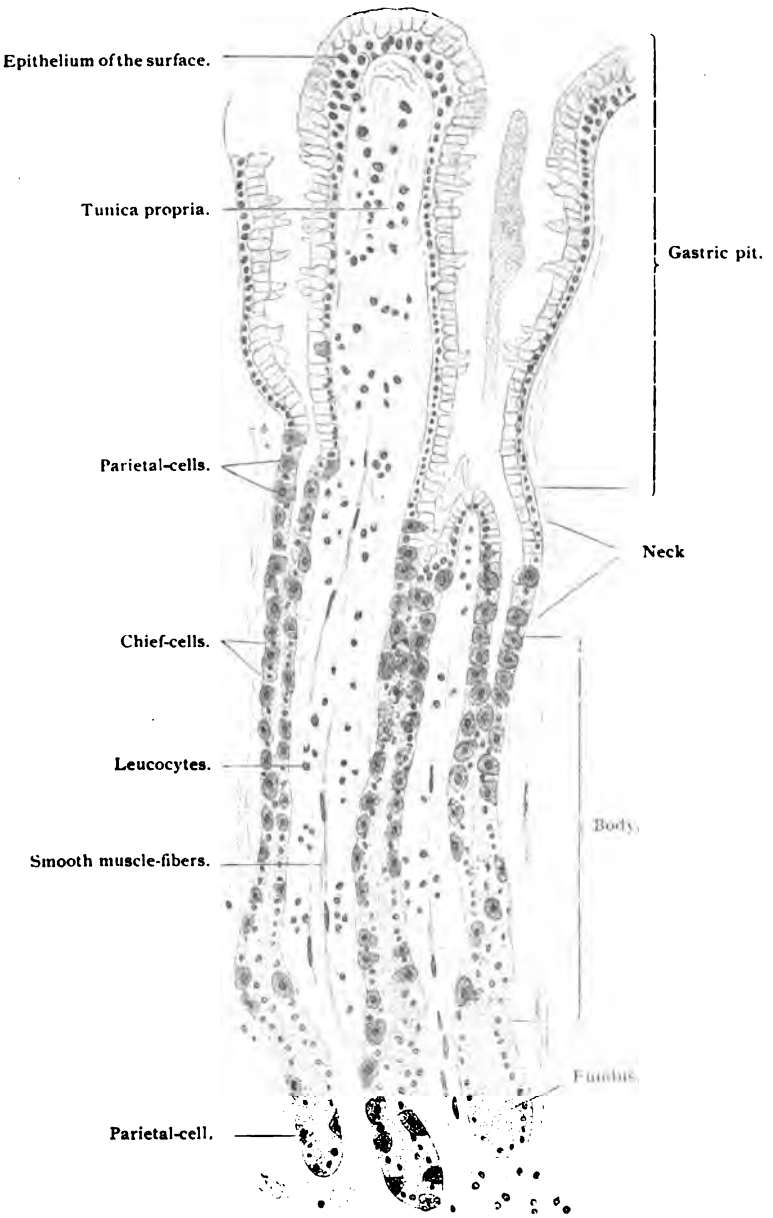



FIG. 154.—VERTICAL SECTION OF THE MUCOUS MEMBRANE OF THE CARDIAC END OF A HUMAN STOMACH. × 220. Techn. No. 102.

marked by their affinity for anilin dyes, with which they react intensely. The two kinds of cells are not equally distributed; the chief-

cells form the principal portion of the gland-fundus, the parietal-cells are irregularly distributed, but are especially numerous in the neck and the body of the tubule. Here they lie in rows beside the chief-cells, but

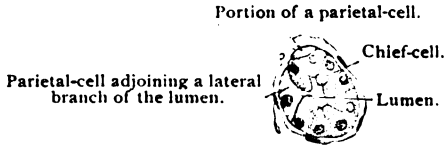



FIG. 155.—TRANSVERSE SECTION OF A HUMAN FUNDUS GLAND. × 240. Techn. No. 102.

toward the fundus they are pressed to the periphery, without, however, being shut off from the lumen, with which they communicate by a short

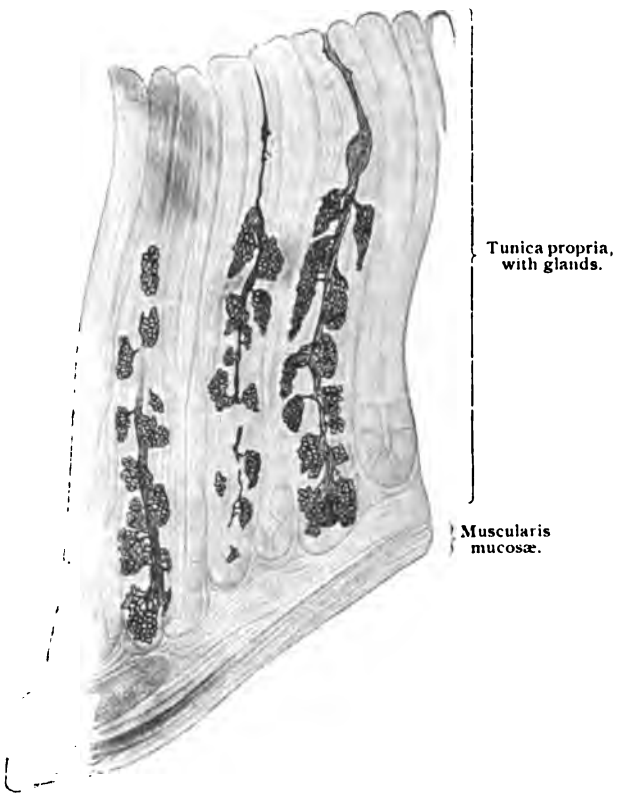



FIG. 156.—CROSS-SECTION THROUGH THE MUCOUS MEMBRANE OF THE FUNDUS OF STOMACH OF A MOUSE (DURING DIGESTION). × 234. In the gland on the right the entire system of canaliculi, in the two other glands only a portion of the same, is silvered. The "baskets" formed by the secretory capillaries can be distinguished. Techn. No. 119.

lateral canal extending between the chief-cells from the lumen to the parietal-cells (Fig. 155). This lateral canal is the only one of the system

of minute canaliculi belonging to the parietal-cells (but not to the chief-cells) that can be seen in ordinary preparations. By the aid of Golgi's reaction, which also "blackens" secretion, it may be seen that from the

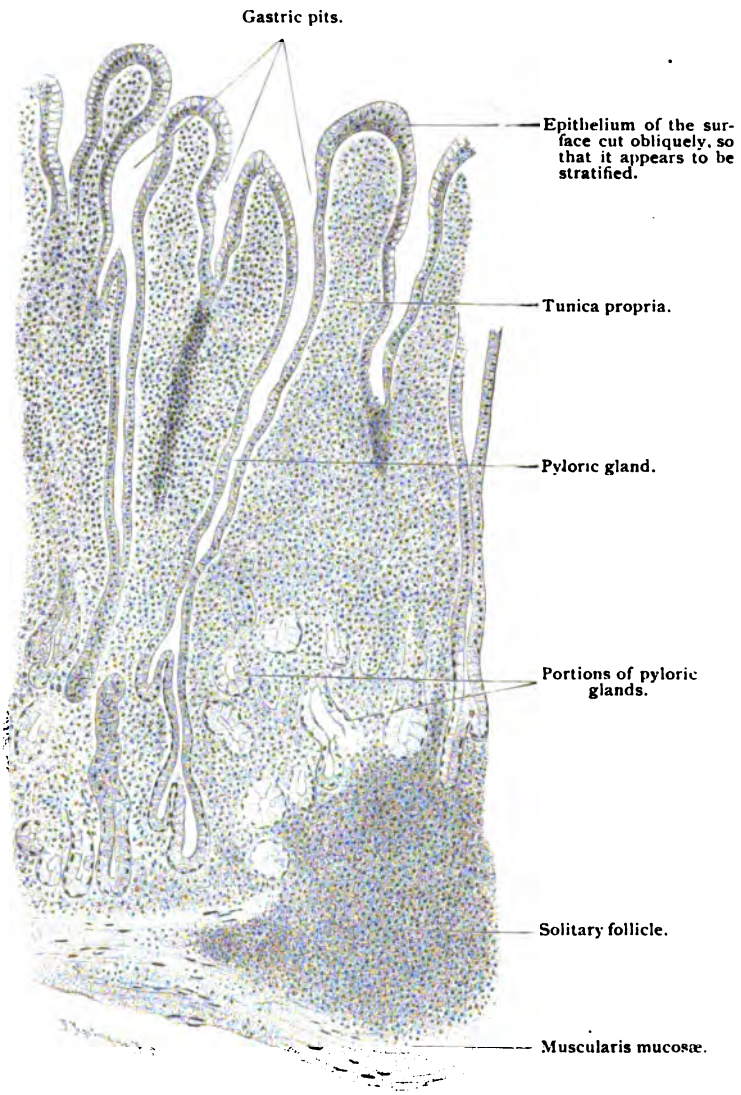



FIG. 157.—VERTICAL SECTION OF THE HUMAN PYLORIC MUCOUS MEMBRANE. × 50. Techn. No. 102 *b*.

axial or chief lumen of the fundus glands transverse canaliculi emerge, that divide and either terminate in free branches or anastomose with one another and form a narrow-meshed network of "secretory capillaries," that surrounds each parietal-cell like a basket or spreads out within the

cell itself. (Fig. 19 and Fig. 156.) The secretion discharged from all sides of the cell passes into the secretory capillaries, then into one or more short lateral canals, and finally into the lumen of the gland.

The *pyloric glands* are furnished almost throughout with columnar cells * containing a spherical nucleus situated near the base of the cell, which in the intermediate zone, that is, the border zone between the pyloric and the fundus mucous membrane, very closely resemble the chief-cells, to which they have been compared.

The foregoing description applies to the stomach as it appears when fasting; during digestion the parietal-cells are larger, the chief-cells, as well as the cells of the pyloric glands, are darker, the nuclei of the latter are pushed nearer to the middle of the cell, and the secretory capillaries expanded with increased contents are wider than in the fasting organ.

The *muscularis mucosæ* consists of smooth muscle-fibers arranged in two or three layers superposed in different directions, from which single strands branch off and ascend vertically between the gland-tubules (Fig. 154).

The *submucosa* is composed of loosely-united connective-tissue bundles and elastic fibers and occasionally contains small clusters of fat-cells.

The *muscular coat:* it is only in the pyloric region that two separate layers of smooth muscle-fibers can be distinguished, a thicker inner circular and a thinner outer longitudinal layer. In the other regions of the stomach the arrangement of the muscle-tissue is very complicated owing to the extension of the muscular strata of the esophagus to the stomach, as well as to the curving of the organ that ensues in the course of development; sections exhibit bundles of fibers extending in every possible direction (Fig. 153).

The *serous coat* will be described with the peritoneum.

For the vessels and nerves see p. 236 and p. 238.

## THE INTESTINES.

The wall of the intestines, like that of the stomach, is composed of three tunics, a mucous, a muscular, and a serous.

The *mucosa* is thrown into circular folds, the valvulæ conniventes, well marked in the upper part of the small intestine, the object of which is to increase the superficial extent of the membrane. In addition to these readily perceptible plications there are still other contrivances

* In man isolated parietal-cells are found; in animals, *e. g.*, the dog, a few dark conical cells occur, that owe their appearance to the compression exerted by neighboring cells.

serving a similar purpose, that stand at the limit of macroscopic perception. These are the minute elevations and depressions of the mucous membrane. The former, the *villi*, are present only in the small intestine, in the large intestine of man they are wanting; they are processes about one mm. high, in the duodenum of leaf-like, in the remainder of the small intestine of cylindrical form. The depressions begin at the pylorus and are found throughout the whole length of the intestine. They exist in their most primitive form in fishes and originate in longitudinal parallel

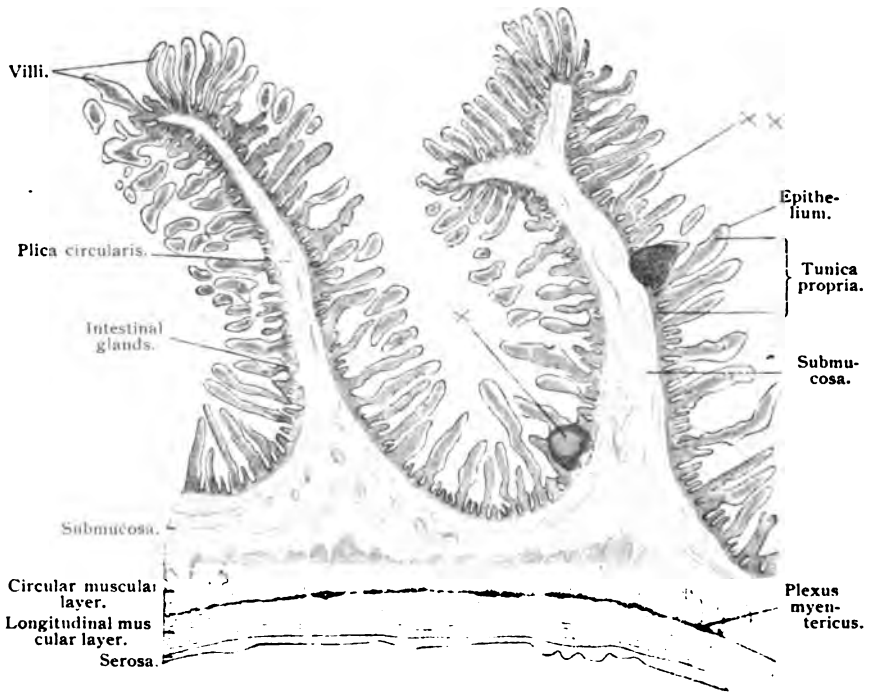



FIG. 158.—LONGITUDINAL SECTION OF THE JEJUNUM OF ADULT MAN. × 16. The circular fold (valvula conniventis) on the right supports two small solitary nodules, that do not extend into the submucosa and of which the left exhibits a germinal center, X. The epithelium is slightly loosened from the connective-tissue core of many of the villi, so that a clear space, XX, exists between the two. The isolated bodies lying near the villi (more numerous to the left of the valvulæ conniventes) are partial sections of villi that were bent, therefore not cut through their entire length. Techn. No. 105.

folds of the mucous membrane connected by small transverse folds. In vertical sections these shallow depressions appear as short, wide sacks, called *crypts*. In mammals the crypts are deeper, their lumen narrower, and in rows close beside one another they have the appearance of simple tubular glands; but they could only be regarded as such if the epithelial-cells lining them produced a specific secretion, which is not the case. However, the name *intestinal glands* (Lieberkühn) has been retained. Whether the isolated granular cells that occur in the fundus of the crypts are gland-cells, is a question.

The *mucous membrane* consists of an epithelium, a tunica propria, a muscularis mucosæ, and a submucosa. The epithelium, which clothes

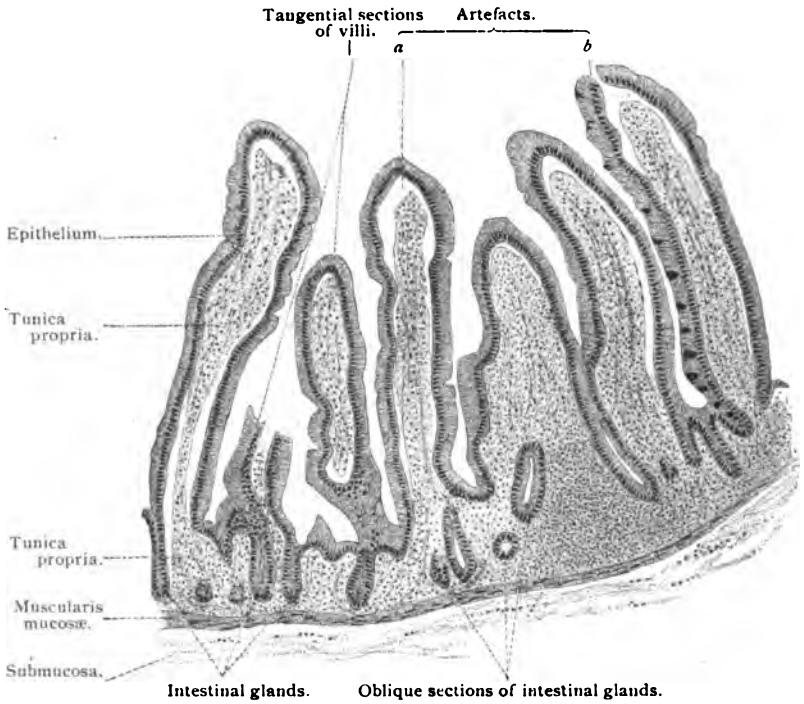



FIG. 159.—SECTION OF THE MUCOUS MEMBRANE OF THE JEJUNUM OF ADULT MAN. × 80. The empty space, *a*, between the tunica propria and the epithelium of the villi is an artificial product, the result of the shrinking action of the fixing fluid. Not infrequently within the space lie cells that have been pressed out of the tunica propria. In its retraction the epithelium often tears and then the villus appears to have an opening, *b*, at its apex. The goblet-cells have been drawn on one side of the villus to the right. Techn. No. 105.

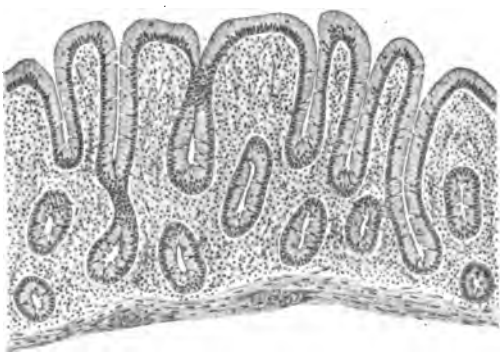


FIG. 160.—SECTION OF THE MUCOUS MEMBRANE OF THE LARGE INTESTINE OF ADULT MAN, SHOWING THE INTESTINAL GLANDS (CRYPTS OF LIEBERKÜHN). × 80. (Schaper.) Techn. No. 108.

the entire free surface, envelopes the villi and lines the crypts, is a simple columnar epithelium, the elements of which in their mature condition

consist of a granular protoplasm containing numerous resorbed fat-particles, a usually oval nucleus, and a cell-membrane. On the free surface of the cells there is a sometimes homogeneous, sometimes finely-striated *cuticular border* characteristic of the intestinal epithelium.

The regeneration of the epithelium takes place only in the intestinal crypts, where by mitotic division new cells are constantly formed, which gradually move upward and replace the cells that disintegrate on the upper surface of the mucous membrane. Therefore the youngest generation of epithelial-cells is found in the crypts, the oldest on the free upper surface, in the small intestine on the apices of the villi. *Goblet-cells* in extremely variable numbers occur in the intestinal epithelium; they possess an elliptical, not infrequently a chalice-like form; the upper portion, that directed toward the surface of the intestine, undergoes different degrees of distention as the protoplasm is transformed into mucus, the nucleus with the remainder of the unaltered protoplasm lies

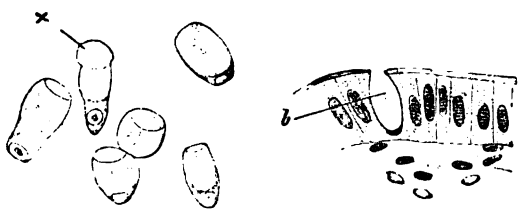



FIG. 161.—INTESTINAL EPITHELIUM. × 560. A. Isolated goblet-cells of rabbit. x, Escaping mucus. Techn. No. 104 *b*. B. From a section of the mucous membrane of the human intestine. *b*, A goblet-cell between columnar cells. Techn. No. 102.

at the base of the cell; a cuticular border is wanting, in place of which a sharply-defined circular orifice is found, through which the mucus is poured out on the surface (Fig. 161, A). The goblet-cells are derived from the ordinary epithelial-cells of the intestine. In proper conditions each young intestinal epithelial-cell may assume the functions of a goblet-cell.*

The separate phases of secretion appear in regular sequence and so that the later phases are always to be seen on the apices of the villi or near the upper surface of the mucous membrane, the initial phases in the intestinal crypts (Fig. 162).

In the crypts of the small intestine the number of goblet-cells is proportionately less than in the large intestine; this is explained by the fact that the young epithelial-cells of the crypts move more rapidly to the

* In regard to the mode in which the goblet-cells produce and discharge secretion, see p. 69.

surface, the greater superficies of the small intestine, so much increased by the villi, necessitating a greater supply of young cells to replace those that disintegrate on the surface; the elaboration of mucus often does not take place in the crypts, but first begins in the cells on the villi. In the large intestine, where the villi are absent, the passage to the surface takes place slowly and the cells have time to produce secretion during their sojourn in the crypts. Out of this arose the misconception that the crypts

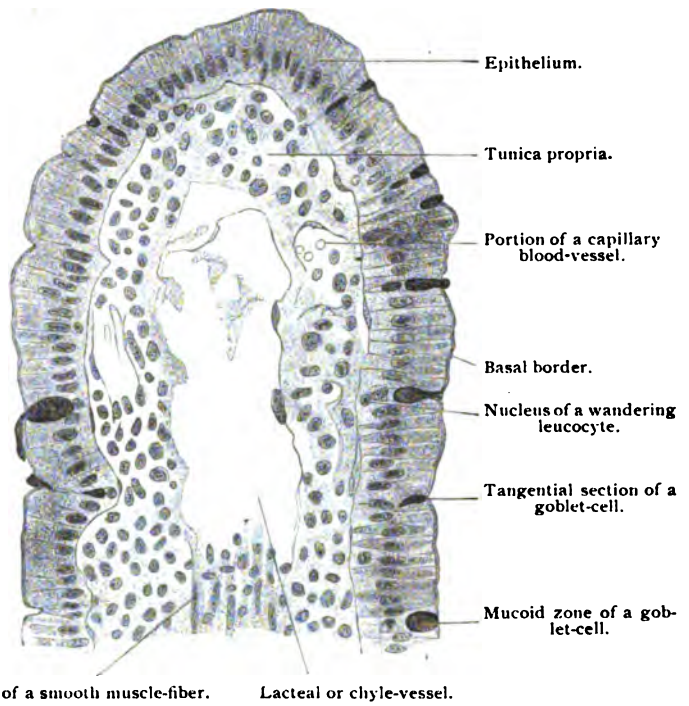



FIG. 162.—LONGITUDINAL SECTION THROUGH THE APEX OF THE VILLUS OF A DOG. × 360. The goblet-cells contain the less mucus the nearer they lie to the summit of the villus. Techn. No. 106.

of the small intestine produced a serous fluid; those of the large intestine a mucoid secretion.

Between the epithelial-cells migratory leucocytes from the underlying tunica propria are found in varying numbers.

The *tunica propria* chiefly consists of fibrillar and reticular connective tissue that contains an extremely variable number of leucocytes. Owing to the numerous crypts present the tunica propria of the large intestine is confined to the spaces between and to a narrow zone below the tubules, as in the stomach; throughout the small intestine the tunica propria extends into the villi.

The *muscularis mucosæ* consists of an inner circular and an outer longitudinal layer of smooth muscle-fibers. Fibers derived from the muscularis mucosæ extend within each villus nearly to its apex. Their contraction effects a shortening of the villus (cf. Techn. No. 105).

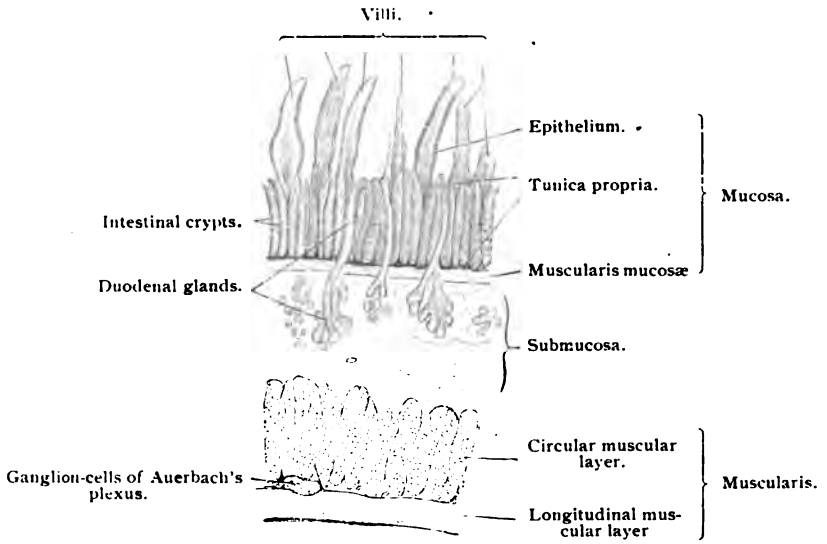



FIG. 163.—LONGITUDINAL SECTION THROUGH THE DUODENUM OF A CAT. × 30. The epithelium has become loosened from the connective tissue of the villus on the extreme left. The two villi at the extreme right are cut obliquely. The epithelium has fallen from the middle villus, so that the connective-tissue core lies exposed. The serosa is represented by a line beneath the longitudinal layer of the muscular coat. Techn. No. 103.

The *submucosa* consists of loose fibrous connective tissue and in the upper half of the duodenum contains branched simple tubular glands, the *duodenal glands* (Brunner), from 0.2 to 3.4 mm. in size. The excretory ducts of these glands are clothed with columnar cells, pierce the muscularis mucosæ, and run in the tunica propria parallel with the crypts of Lieberkühn. The walls of the tubules are formed of columnar gland-cells and a structureless membrana propria.

## THE LYMPH-NODULES.

It has been previously mentioned that the tunica propria of the mucous membrane contains leucocytes or lymphoid-cells in variable numbers, occurring either as diffuse adenoid tissue or as circumscribed masses from 0.5 to 2 mm. in size. The latter are lymph-nodules, which occur either singly as the *solitary nodules* or in groups as the *agminated nodules*.

The *solitary nodules* ("solitary follicles") vary greatly in number in the gastric mucous membrane, they are more numerous in the intes-

tines. They usually possess an oval form and in the beginning of their development always lie in the tunica propria, close under the epithelium, with their base directed toward the muscularis mucosæ. With advancing growth (in cats at birth) they break through the muscularis mucosæ and expand in the submucosa, where the loose tissue offers but little resistance. The part of the nodule lying in the submucosa has a spherical outline and soon becomes considerably larger than that within the tunica propria. The completed solitary nodules, therefore, are in general pear-shaped, with the small end turned toward the epithelium.

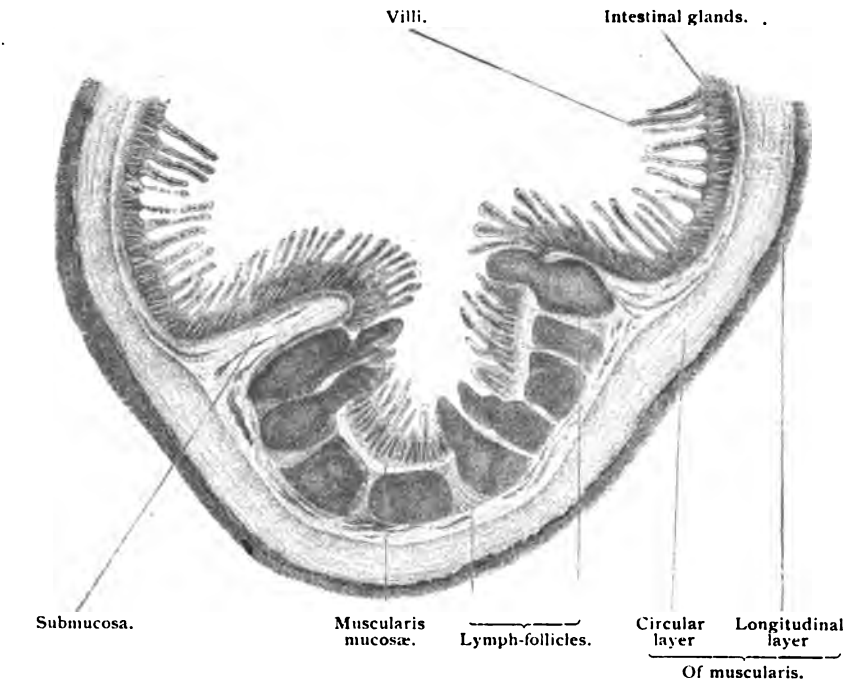



FIG. 164.—TRANSVERSE SECTION OF A PATCH OF PEYER OF THE SMALL INTESTINE OF A CAT. The crests of four nodules were not within the plane of the section. × 10. Techn. No. 107.

Where the nodules are situated the villi are wanting and the crypts are pushed aside. The solitary nodules are composed of adenoid tissue and usually contain a germinal center. The young leucocytes formed in them in part pass into the neighboring lymph-vessels and in part wander through the epithelium into the intestine. The columnar epithelium covering the apex of the nodules contains wandering leucocytes (Fig. 165).

The *agminated nodules* (patches of Peyer) are groups of from ten to sixty nodules that lie side by side, never over one another, each of which

has the structure of a solitary nodule. Occasionally the outline of an individual nodule is altered by the pressure of adjacent nodules (Fig. 164). They principally occur in the lower portion of the small intestine, either isolated from one another or transformed in a mass of diffuse adenoid tissue, in which case only the germinal centers can be distinguished. This is not infrequently the case in the vermiform process of man. The transition of the mucous membrane of the rectum, characterized by the large intestinal crypts, into that of the anal canal occurs at the

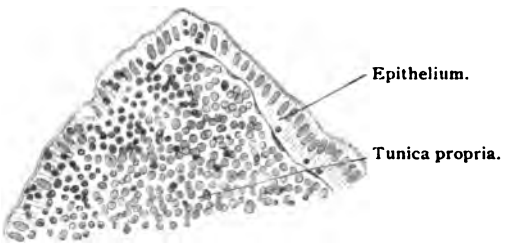



FIG. 165.—FROM A SECTION OF THE SMALL INTESTINE OF A SEVEN-DAYS'-OLD KITTEN. × 250. Crest of a solitary follicle. The epithelium on the left contains many wandering leucocytes. The epithelium on the right contains but three leucocytes. Techn. No. 107.

upper end of the columnæ rectales (Morgagni); the crypts cease and instead of the simple cylindrical epithelium a thick, stratified squamous epithelium appears, which covers papillæ containing blood-vessels.

The *muscular layer* of the intestine consists of an inner robust circular and an outer thinner longitudinal stratum of smooth muscle-fibers. In the large intestine the longitudinal muscular layer is well developed only at the folds corresponding to the intervals between the sacculi; between these folds it is extremely thin.

The structure of the *serosa* will be described with the peritoneum (p. 254).

### THE BLOOD-VESSELS OF THE STOMACH AND OF THE INTESTINES.

The blood-vessels of the stomach and of the large intestine have a precisely similar distribution, which is modified in the small intestine by the presence of the villi. In the stomach and in the large intestine the entering arteries first give off small branches to the serosa, then pierce the muscularis, which they supply, and then in the submucosa form a network extending parallel to the surface. From this small twigs ascend through the muscularis mucosæ and in the tunica propria at the base of the glands form another network parallel to the surface. Fine capillaries (from 4.5 to 9 $\mu$ wide) arise from the latter, which form plexuses around the glands and pass into wider capillaries (from 9 to 18 $\mu$), which latter

form a subepithelial plexus, that wreath-like lies about the mouths of the glands. Venules take their origin from the wide capillaries, pass vertically down between the gland-tubules and open into a venous plexus lying parallel to the surface in the tunica propria; in their further course the veins run alongside the arteries. The veins arising from the venous plexus in the submucosa are furnished with valves to the point where they open into the collecting veins approaching the intestine along parallel paths. The remaining branches and the trunk of the portal vein are without valves.

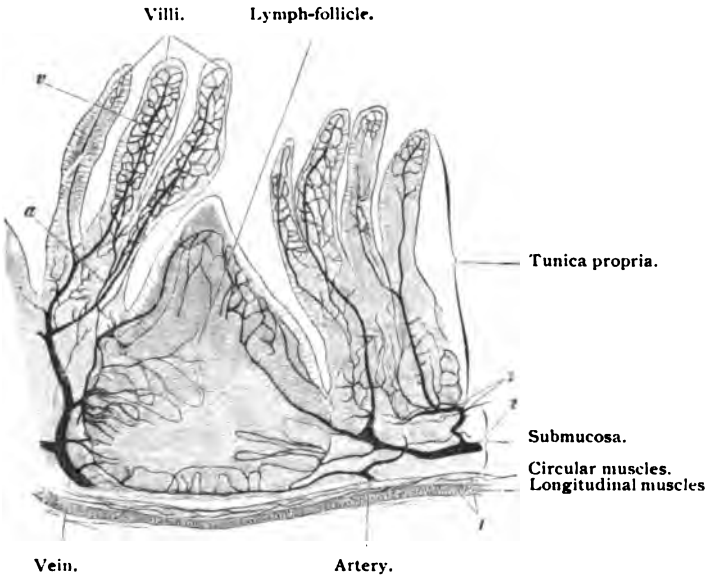



FIG. 166.—FROM A CROSS-SECTION OF THE INJECTED SMALL INTESTINE OF A RABBIT. × 50. The lymph-nodule is sectioned so that in the upper half the superficial capillary network is visible, in the lower half, the capillary loops occurring within the interior of the nodule. The section is thick and unstained, and the crypts of Lieberkühn cannot be distinguished. 1. The network of blood-vessels within the muscularis; 2, within the submucosa; 3, within the tunica propria. Techn. No. 110.

In the small intestine only the arteries supplying the crypts are distributed in the same manner as in the large intestine. The villi are provided with one artery (several when the villi are broad), which lies opposite the vein; from the former capillaries arise that lie close under the epithelium and, obliquely or vertically to the long axis of the villus, pass into the veins.* The further course of the veins is the same as in the large intestine.

* The distribution is the same in the dog, but in the rabbit and the guinea-pig the arteries going to the villi break up into fine branches that run to the base of the villus and then form a capillary network that lies close under the epithelium (Fig. 166, *a*). At the summit of the villus the capillaries open into a small venous trunk that in the course of its vertical descent takes up the capillaries surrounding the mouths of the glands (Fig. 166, *v*).

The duodenal glands are enveloped in a capillary plexus supplied by the blood-vessels of the submucosa.

The lymph-nodules are surrounded by a superficial capillary network, from which fine capillaries extend into the interior; often these do not penetrate to the center, which is then without blood-vessels (Fig. 166).

### The Lymph-vessels of the Stomach and of the Intestines.

The lymph- (chyle) vessels of the stomach and of the large intestine begin in the mucous membrane as blind capillaries, about 30 $\mu$ wide, and descend between the gland-follicles. In the mucous membrane of the small intestine the lymph-vessels begin in the axis of the villi; in cylindrical villi they are simple (in leaf-shaped villi multiple) canals (from 27 to 36 $\mu$ wide) closed at their upper ends, the lymph-radicles or lacteals. All these vessels descend to a narrow-meshed capillary plexus lying at the base of the glands and extending parallel to the surface, which communicates by numerous anastomoses with a wide-meshed horizontal plexus in the submucosa; the lymph-vessels proceeding from this network are provided with valves; they penetrate the muscular coat and take up the vessels of a plexus lying between the circular and the longitudinal muscular strata, called the intramuscular lymphatic plexus, which takes up the numerous lymph capillaries of both muscular layers. The vessels then run beneath the serosa to the mesentery and pass onward between its folds.

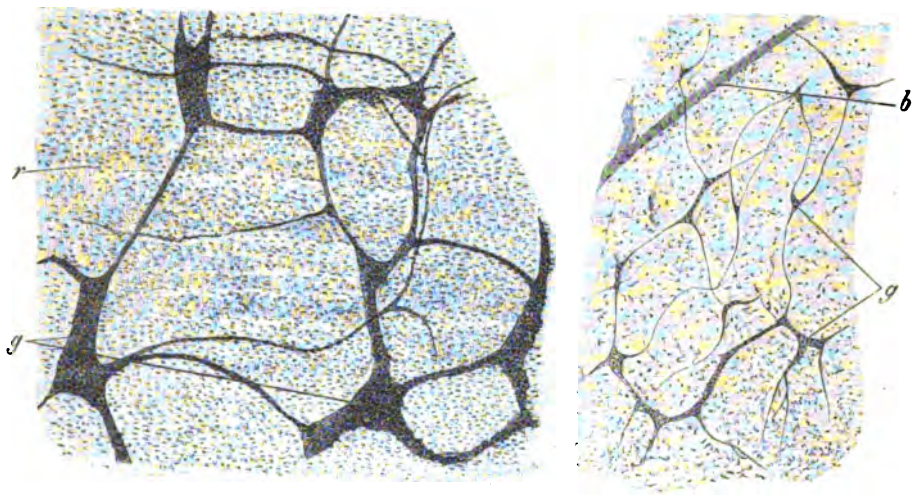
In certain localities the course of the lymph-vessels in the mucosa is modified. The nodules of the patches of Peyer never contain lymph-vessels. They press aside the capillaries, which run in the interstices between them, constantly decreasing in number but increasing in caliber. It is probable that the lymph-sinuses of the rabbit (p. 125, remark) are nothing else than such immensely-widened, flattened capillaries.

### The Nerves of the Stomach and of the Intestines.

The numerous nerves, mainly consisting of gray fibers, form a plexus beneath the serosa, then pierce the longitudinal layer of the muscular tunic and between this and the circular layer are arranged in a conspicuous network, the *intramuscular ganglionic plexus* (*plexus myentericus*) (Auerbach); numerous groups * of multipolar ganglion-cells are found along the course of the nerves, usually at the nodal points of the network, the meshes of which are angular or elliptical. From this network bundles of gray fibers are given off usually at right angles, that in part

* The groups—small ganglia—behave like the sympathetic ganglia in general.

supply the longitudinal and circular strata of the muscular tunic, while another portion pierces the latter and enters the submucosa. In the muscular coat the nerves form a rich rectangular-meshed network, from which nerve-fibers turn aside and after repeated division approach the muscle-fibers, on which (not within) they terminate in free club-shaped endings. The nerves in the submucosa form a delicate plexus, the *plexus submucosus* (Meissner), the meshes of which are narrower and groups of




A B

FIG. 167.—A. SURFACE VIEW OF THE PLEXUS MYENTERICUS OF AN INFANT. × 50. *g*, Groups of ganglion-cells; *r*, layer of circular muscle-fibers, recognized by their rod-shaped nuclei. Techn. No. 111 *a*.

B. SURFACE VIEW OF THE PLEXUS SUBMUCOSUS OF THE SAME INFANT. × 50. *g*, Groups of ganglion-cells; *b*, blood-vessel shimmering through the overlying tissue. Techn. No. 111 *b*.

ganglion-cells smaller. From this spring numerous fibers which enter the tunica propria and in part weave a nervous net about the glands, in part enter the villi, where they terminate free in the parenchyma or close beneath the epithelium, without connection with the epithelial-cells.*

A network corresponding to the intramuscular ganglionic plexus also occurs between the layers of the muscular coat of the esophagus.

## THE SALIVARY GLANDS.

The salivary glands are the submaxillary, sublingual, and parotid glands, and the pancreas. They are compound tubular glands, which

* Spindle-shaped or stellate elements have been described as nerve cells of the intestinal plexuses, the processes of which form a plexus surrounding the blood- and lymph-vessels. They do not stand in any relation to the above-described plexuses, nor can any nerve-processes be distinguished on them, so that their nature is still uncertain.

elaborate either a mucous or a richly-albuminous serous fluid, or both the mucous and the serous secretion. Accordingly we distinguish: (1) *mucous salivary glands* (sublingual in man, the rabbit, dog, and cat; submaxillary in the dog and cat); (2) *serous salivary glands* (the parotid in man, the rabbit, dog, and cat; submaxillary in the rabbit, and the pancreas); (3) *mixed salivary glands* (submaxillary in man, the ape, guinea-pig, and mouse).

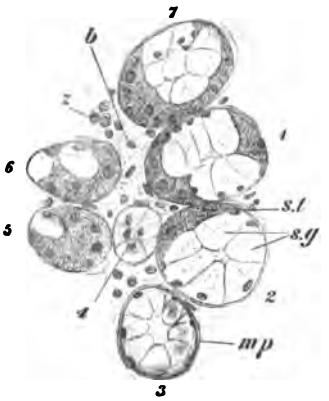



FIG. 168.—FROM A THIN CROSS-SECTION OF HUMAN SUBLINGUAL GLAND. × 240. Of the seven tubules represented, only three (1, 2, 3) are sectioned so as to be suitable for study. In 2 are six cells loaded with secretion (*s.g*); and two empty cells (*s.l*) are crowded to the periphery, where they form a "crescent." In 3 all the cells are filled with secretion, and have deeply-stained contents; 4, tangential section of a similar tubule. 5, 6, 7, oblique sections of tubules like 1 and 2, which show the crescents, but not the lumen of the gland. *mp*, Membrana propria. *b*, Connective tissue with numerous leucocytes, *z*. Techn. No. 112.

*The Sublingual Gland.*—The excretory duct, *ductus sublingualis* (Bartholin), consists of a two-layered cylindrical epithelium and fibro-elastic tissue. It is continued as the intralobular or mucous tubes, the low columnar epithelium of which exhibits the characteristic striation (Fig. 170, *A*) only in a few places. Intercalated tubules cannot be demonstrated with certainty, it is much more probable that the mucous tubes pass directly into the terminal compartments. The latter are composed of a membrana propria and of mucous cells. The membrana propria is formed by stellate connective-tissue cells; the empty glandular cells occur in groups, the "demilunes" therefore appear very large (Fig. 168, 1, 2). The connective-tissue between the tubules and the lobules is rich in leucocytes.

*The Parotid Gland.*—The excretory duct, *ductus parotideus* (Stenon), is distinguished by its broad, compact membrana propria close beneath the epithelium, but otherwise is like that of the sublingual gland. It divides and passes into the *intralobular* tubes, the columnar cells of which at the base exhibit distinct vertical striation. Following these are the intercalated tubules, which are lined by elongated, often spindle-shaped, cells. The intercalated tubules continue to the terminal compartments, which consist of a delicate membrana propria with stellate connective-tissue cells and of cubical serous glandular cells. In a state of exhaustion the cells are small, dark, and granular; in a state of activity they appear larger and somewhat clearer.

*The Submaxillary Gland.*—The excretory duct, *ductus submaxillaris* (Wharton), likewise possesses a two-layered columnar epithelium, a

connective-tissue layer rich in cells, and outside of this a thin stratum of longitudinally-disposed muscle-fibers; it continues as the intralobular tubes lined with characteristic epithelium* (Fig. 170, A), which pass into

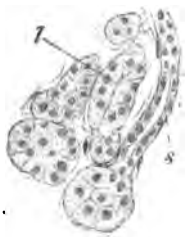



FIG. 169.—FROM A THIN SECTION OF HUMAN PAROTID GLAND. × 240. *s*, Intercalated tubule; the outlines of the cells cannot be distinguished. The very narrow lumen of the gland-tubule is seen only at *l*; the remaining gland-tubules are cut obliquely. Techn. No. 112.

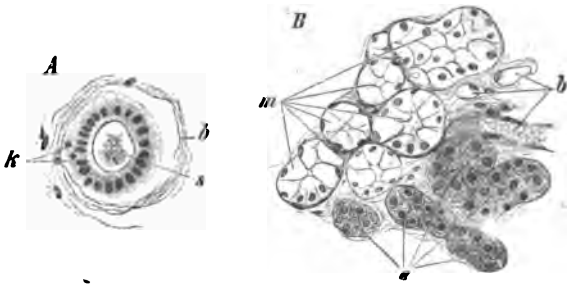



FIG. 170.—FROM A THIN SECTION OF HUMAN SUBMAXILLARY GLAND. × 240. *A*. Cross-section of a salivary tube; the epithelial cells on the right are partially loosened from the surrounding connective tissue, *b*; on the same side the striation in the outer zone of the cells is best seen; *k*, nuclei of wandering leucocytes; *s*, secretion. *B*. Tubules (*m*) with mucous gland-cells showing four lumina; *e*, tubules with serous gland-cells showing one lumen; *b*, blood-vessels, of which the lowermost is cut longitudinally and contains colored blood-corpuscles. Techn. No. 112.

the short intercalated tubules clothed with cubical cells. The latter lead into the end-pieces, which are clothed either with serous gland-cells (as in the parotid) or with mucous gland-cells and demilunes.

*The Pancreas.*—The excretory ducts, *ductus pancreaticus* (Wirsung)

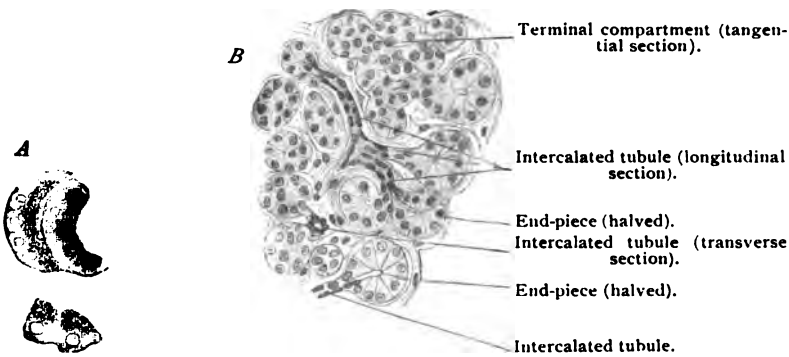



FIG. 171.—*A*. GLAND-CELLS FROM THE PANCREAS OF A CAT. × 560. Above, groups of cells as they usually appear; below, two isolated cells. *B*. FROM A CROSS-SECTION OF THE PANCREAS OF AN INFANT. × 240. Techn. No. 113.

and *ductus pancreaticus accessorius* (Santorini), are formed of a simple columnar epithelium and of fibrous connective tissue, which latter is denser beneath the epithelium, looser toward the periphery. The walls

* It contains a yellow pigment, that is visible in preparations fixed with alcohol.

of the main excretory duct and its larger branches contain minute mucous glands. Intralobular tubes with their characteristic epithelium are wanting. The branches of the excretory duct continue directly into the intercalated divisions; the columnar epithelial-cells of the former steadily diminish in height and eventually pass over into the flattened cells, placed parallel to the long axis, of the intercalated tubules. These tubules are very long and narrow; toward the end-pieces they divide and then abruptly terminate at the epithelium of the terminal pieces.* This epithelium consists of short cylindrical or conical cells, which are characterized by the highly-refracting granules—"zymogen granules"†—occupying the zone adjoining the lumen and are thus distinguished from all other glandular cells (Fig. 171, *A*, and Fig. 172). The clearer peripheral zone of the cell contains the round nucleus. The granular and clear divisions of the cell vary in proportionate extent with

FIG. 172.—TRANSVERSE SECTION OF A GLAND-TUBULE OF THE PANCREAS OF NECTURUS; showing zymogen granules. × 400.—(*Schaper.*)

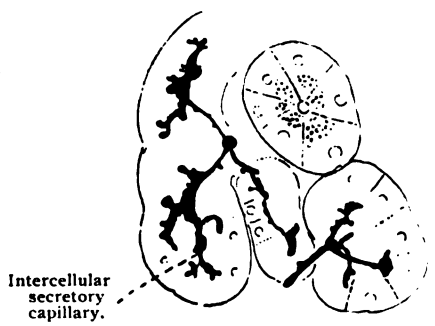



FIG. 173.—FROM A SECTION OF THE PANCREAS OF ADULT MAN. × 320. Techn. No. 119.

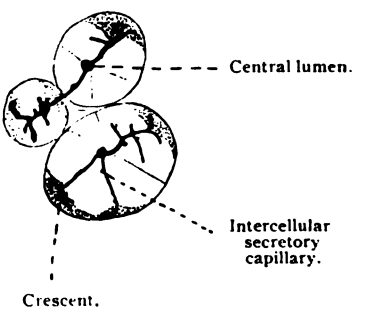



FIG. 174.—FROM A SECTION OF THE SUBMAXILLARY GLAND OF A DOG. × 320. Techn. No. 119.

the functional state of the cell. In the beginning of digestion the granules disappear and the clear belt becomes deeper. Subsequently the

* Continuations of the epithelium of the intercalated tubules into the interior of the terminal pieces form the so-called "centroacinal cells," which as squamous elements lie upon the inner surface of the epithelial-cells of the end-pieces.

† These granules are not artefacts, as are so many other granules in cells, which are produced by fixation in sublimate or nitric acid. The zymogen granules can be seen in the cells of the fresh pancreas.

granular zone increases to such an extent that it occupies nearly the whole of the cell. In a fasting state the two zones are of equal size.

In glands treated by the method of Golgi, the secretion often stains and the duct-system in its entire extent appears black. The secretory capillaries going from the central lumen of the terminal compartments may then be seen (Fig. 173 and 174) running between the gland-cells; they do not quite reach to the membrana propria and without anastomoses terminate in free branches. Their terminal ends possibly lie within the gland-cells (cf. p. 74).

The *blood-vessels* of the salivary glands are very richly developed. The arterial stems, as a rule, run along side the main excretory duct, where they divide into numerous branches which pass between the lobules and finally penetrate within the latter, break up into capillaries and form close networks around the tubules. The capillaries lie in immediate proximity to the gland-cells. The larger veins follow the course of the arteries.

With regard to the *lymph-vessels* little is known with certainty. The interfascicular clefts between the lobules and the tubules have been described as lymph-channels.

The salivary glands are profusely supplied with plexuses of medullated and nonmedullated *nerves*, along the course of which microscopic groups of ganglion-cells occur. The fine nonmedullated nerve-fibers partly ramify in the walls of the blood-vessels, partly form an "epilemmal" plexus lying immediately upon the membrana propria of the gland-tubules; from this delicate filaments arise which pierce the membrana propria and as hypolemmal fibers terminate in short, varicose, simple or branched ends, which lie upon the gland-cells.*

## THE LIVER.

The liver is a compound tubular gland. On making an incision into a liver or on examining its outer surface, it will be observed that it is divided into irregular polygonal areas, well defined, as in the hog, or poorly defined, as in man and the majority of mammals. These areas are the *lobules* of the liver (incorrectly named acini). Their real form is somewhat like that of a prism with a rounded upper end and a transversely-blunted base (Fig. 175). They are 2 mm. high and 1 mm. broad. Close under the capsule of the liver the lobules often are arranged with their apex looking toward the surface and a section made parallel to the surface will pass through the lobules transversely (Fig. 177); in the

* In other glands (coil glands, mammary glands, tarsal glands) the relations are the same.

interior of the liver the lobules stand in different directions. Each lobule consists of gland-cells and blood-vessels and is separated from neighboring lobules by the *interlobular* connective tissue, which supports the branches of the excretory duct (the hepatic duct), the branches of the portal vein and the hepatic artery, the lymph-vessels and the nerves. The demarcation of the lobules depends on the development of the interlobular connective tissue.

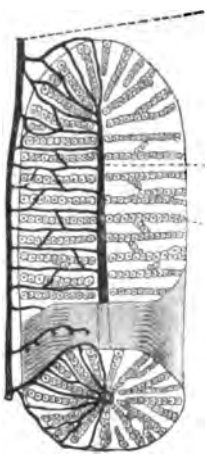



FIG. 175.—SCHEME OF AN HEPATIC LOBULE, represented in transverse section below and, by partial leveling, in longitudinal section above. In the left half the blood-vessels are drawn; in the right half only the cords of hepatic cells. × 20.

The main excretory duct, the *hepatic duct*, and its larger branches consist of a single stratum of columnar epithelium, occasionally containing goblet-cells, and of fibrous connective tissue separated into tunica propria and submucosa. The tunica propria is the carrier of the glands of the bile-duct, chiefly short, pear-shaped follicles lined with mucous gland-cells, and of isolated longitudinally- and transversely-disposed plain muscle-fibers. The *cystic duct*, the *ductus choledochus*, and the *gall-bladder* exhibit the same structure; the tunica propria is elevated into minute anastomosing rugæ and the mucosa is supplemented by a thin layer of interlacing smooth muscle-fibers. The columnar epithelial-cells of the gall-bladder are distinguished by their height (0.05 mm.) from those of the ductus choledochus (0.024 mm.).* The branches arising from the further division of the hepatic duct, the *interlobular bile-ducts*, exhibit thinner walls as they diminish in caliber; the larger consist of simple columnar epithelium and fibro-elastic tissue, the smallest possess only a structureless membrana propria and a simple layer of low epithelial-cells provided with a cuticular border, which as they enter at the margin of the lobules annex themselves directly to the true glandular cells. This transition is very difficult to see and can be distinctly perceived only in sections in which the bile-ducts have been injected or blackened by Golgi's silver method.

The glandular cells of the liver, the *hepatic cells*, are irregular poly-

* The *vasa aberrantia*, or blind bile-ducts, that chiefly occur at the left border of the liver, at the portal fissure, and in the neighborhood of the vena cava, are embryonal remains of liver substance and do not occur in the parenchyma of the organ.

hedral elements consisting of a granular protoplasm and of one or more nuclei; they have no cell-membrane. The protoplasm contains granules of pigment and globules of fat of various sizes, which latter are invariably found in mammalian animals and well-nourished persons. The cells

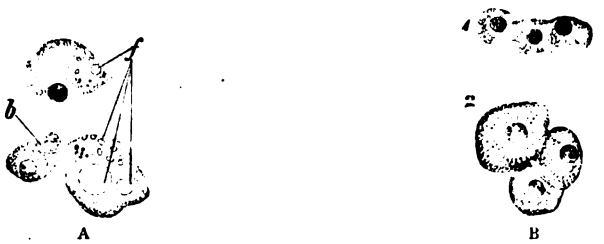



FIG. 176.—LIVER-CELLS OF MAN. ⁄ 560. A. Isolated liver-cells containing smaller and larger fat-drops, *f*; *b*, imprint from contact with a blood-vessel. Techn. No. 114.
B. From a section: 1, exhausted cells; 2, active-cells, filled with secretion. Techn. No. 116.

vary in size from 18 to 26 μ. The appearance of the liver-cells depends, as in other gland-cells, on the phase of functional activity. In a fasting condition they are small, dim, and have indistinct contours; during digestion they are larger, have a clear center, and at the periphery a

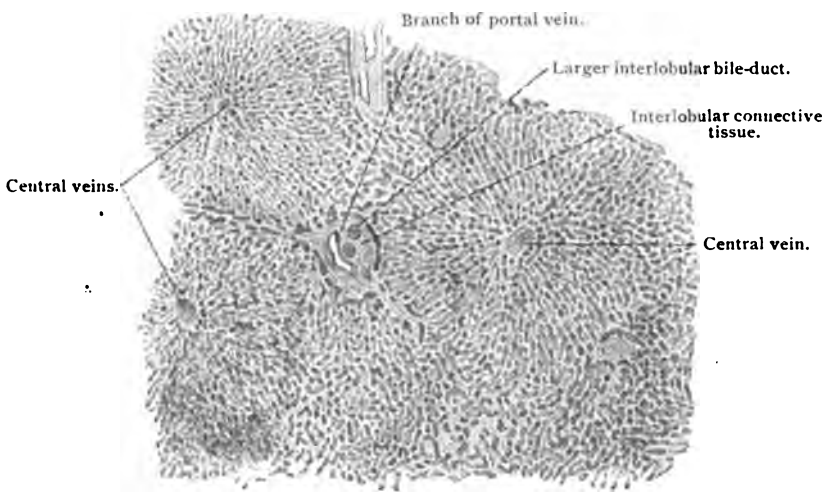



FIG. 177.—FROM A HORIZONTAL SECTION OF HUMAN LIVER. × 40. Three central veins, cut transversely, represent each a center of as many hepatic lobules, that at the periphery are but slightly defined from their neighbors. Below and to the right of the section the lobules are cut obliquely and their boundaries cannot be distinguished. Techn. No. 116.

coarsely-granular zone. In man the two states are frequently exhibited in the same liver (Fig. 176, B).

In the lower vertebrates (amphibians and reptiles) the hepatic cells form typical tubes, but in the higher vertebrates their arrangement is a

very peculiar one and not a trace of tubular structure is to be seen, as might be presupposed from the tubular character of the liver. The cells are united in small trabeculæ or cords, the so-called *cords of liver-cells*, that radially disposed around a small vein (the central vein) situated in the axis of the lobule spread out toward the periphery (Fig. 175 and Fig. 177), and by lateral branches anastomose with neighboring cords of cells. A lumen cannot be distinguished in such cords by the usual methods; it can only be successfully shown by injecting the system of canals from the hepatic duct or by employing Golgi's method, which blackens the

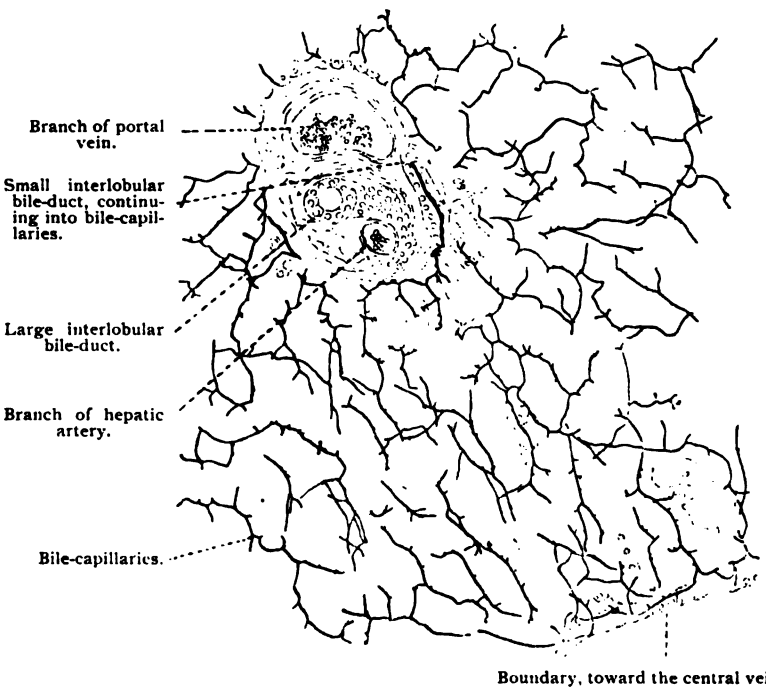



FIG. 178.—TRANSVERSE SECTION OF THE LIVER OF A DOG. × 240. Bile-capillaries blackened according to the method of Golgi. Techn. No. 119.

bile. In such preparations it may be seen that the canal-system (lumen) of the minutest interlobular bile-ducts is continued directly into the lobules and there apparently forms a network with polygonal meshes. In reality there are but few true meshes; the network is simulated by the zigzag course of the bile-canaliculi and the crossing in different planes of the blind lateral twigs with which they are furnished (Fig. 178).

The ramifications of the entire intralobular system of canaliculi appear to have little relation to the branching of the cords of hepatic cells. The latter branch much less than the former and thus, apparently,

the intralobular canaliculi have attained a certain degree of independence, as implied in the name *bile-capillaries*, bestowed upon them. This also accounts for the hitherto always fruitless endeavor to demonstrate a special wall for the bile-capillaries. There can be no wall other than that formed by a modification of the exoplasm of that side of the hepatic cells where the furrow for the bile-capillary is situated; this stratum of the cell protoplasm is continuous with the cuticular border of the epithelial-cells of the interlobular bile-ducts (p. 244).

Thin sections clearly show that the bile-capillaries stand in the same relation to the hepatic cells as the lumina of other glands to the surrounding gland-cells, at least in the main. But nevertheless certain differences exist. The first difference is this, that only a few, usually two hepatic cells suffice to bound the bile-capillaries, while in other glands the lumen is bounded by several cells (Fig. 184). The explanation of this may be found in the conspicuous difference between the diameter of the lumen (the bile-capillary) and that of the hepatic cell; two cells are sufficient to completely surround the lumen. Hence the capillary is formed by the apposition of the furrow-like depressions of two contiguous hepatic cells (Fig. 185).

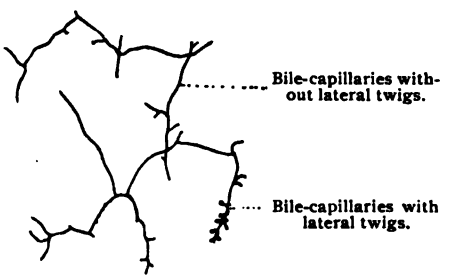



FIG. 179.—FROM A SECTION OF THE LIVER OF A DOG. × 490. Techn. No. 119.

A second difference consists herein, that a distinction between main lumen and secretory capillaries, such as can be made in many glands, is impossible; one cannot say: the hepatic cells present one surface to the main lumen, the other surfaces to the secretory capillaries, one can only say, that the liver-cells present several surfaces to the bile-capillaries. This fact renders intelligible the luxuriant ramification of the latter, despite the fact that relatively few cells are required to circumscribe them.*

Of the *blood-vessels* of the liver, the portal vein assumes the rôle that falls to the artery in other glands, while to the hepatic artery is assigned the subordinate part of the maintenance of the interlobular

* Not infrequently it may be seen that short fine lateral twigs leave the bile-capillaries and terminate in a minute knob-shaped end. The knob corresponds to a small vacuole in the liver-cell, which communicates with the bile-capillary by means of a delicate canal, the minute lateral twig. These undoubtedly are transient formations occurring in connection with certain functional phases; the proof of this I detect therein that entire areas of the system of canaliculi may be free from these knobs, while close beside every capillary is beset with them (Fig. 179).

branches of the bile-ducts, of the portal vein, and of the hepatic veins.

From the branches of the *portal vein*, that because they run in the interlobular connective tissue are called *interlobular veins*, spring numerous capillaries possessing a width of from 10 to 14 $\mu$. They penetrate within the lobules, repeatedly anastomose with one another during their course, and finally empty into a small vein lying in the axis of the lobule, the *central vein* (*intralobular vein*), visible in transverse and longitudinal sections even of the uninjected liver (Fig. 177). The central veins represent the radicles of the hepatic veins and empty into the *sublobular*

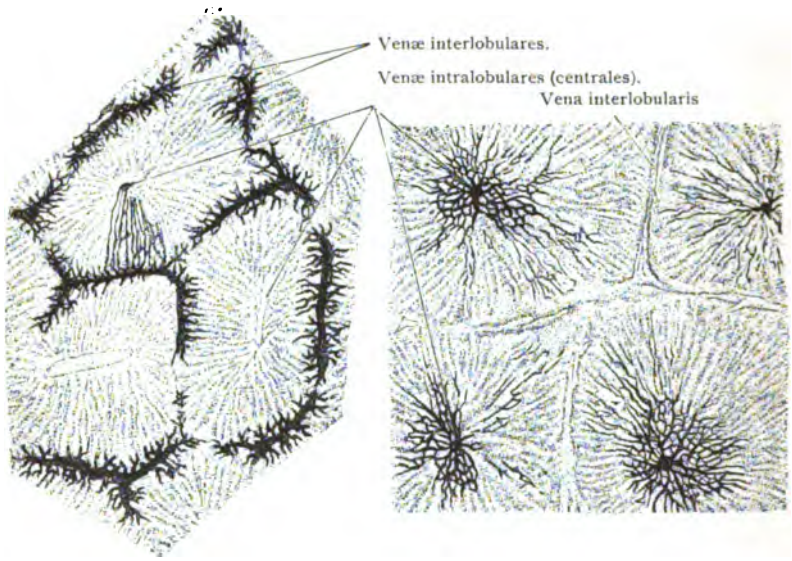



FIG. 180.—HORIZONTAL SECTION OF THE LIVER OF A RABBIT. Injected through the portal vein. × 40. Three hepatic lobules are represented. The injection mass filled only the branches of the portal vein (interlobular veins); in the upper lobule it penetrated to the central vein. Techn. No. 118.

FIG. 181.—HORIZONTAL SECTION OF THE LIVER OF A CAT. Injected through the vena cava inferior. × 40. Four hepatic lobules are shown. The injection mass filled the central vein and the capillaries emptying into it, but did not penetrate to the interlobular veins. Techn. No. 118.

*veins*, which run along the slightly-flattened side, the so-called base, of the hepatic lobules (Fig. 182).

The relation between the portal-capillaries on the one side and the hepatic cells and the bile-capillaries on the other calls for especial consideration. Between the meshes of the portal capillary network the cords of hepatic cells are inserted, consequently the relation of blood-vessels and gland-cells is a very intimate one; sections show that a hepatic cell is in contact with capillaries, not only on one but on several sides (Fig. 183). This is a unique phenomenon, that does not occur in

other glands, where the blood-vessels touch the cells only on one surface, and is only comprehensible when we recall that in cross-sections the lumen (bile-capillary) is bounded by only two cells, while in other

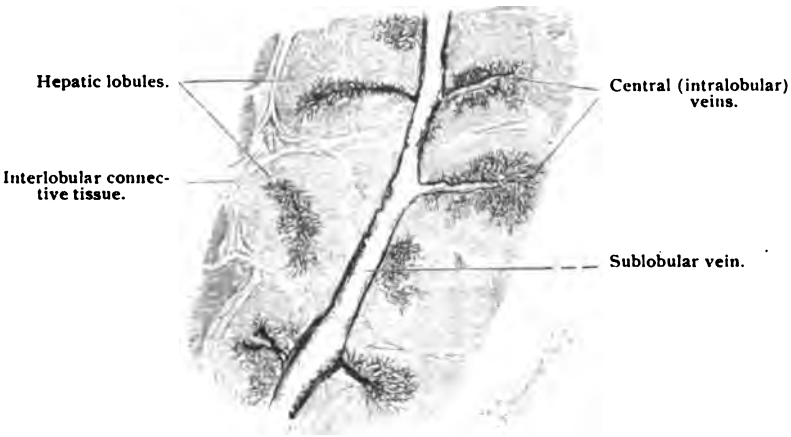



FIG. 182.—FROM A VERTICAL SECTION OF THE LIVER OF A CAT.—Injected through the vena cava inferior. A sublobular vein cut longitudinally; it takes up the central veins. The greater part of the injection mass has fallen out of the wide blood-vessels. × 15. Techn. No. 118.

tubular glands the lumen in cross-section is bounded by six or more cells (Fig. 184). But as in other glands, so also in the liver, the gland-cells are inserted between the lumen on the one hand and the blood-

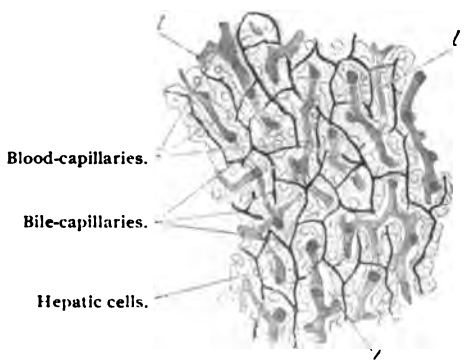



FIG. 183.—FROM A SECTION OF THE LIVER OF A RABBIT. × 240. The portal capillaries were injected with a red mass, the bile capillaries with a blue mass. The hepatic cells are in contact with blood-capillaries on both sides. At a few points the red mass has retracted and given rise to a space (*l*) between the hepatic cells and portal-capillaries. The bile-capillaries are nowhere in contact with portal-capillaries, but are always separated from them by half the breadth of a cell. The dark spots on the portal-capillaries are optical cross-sections of blood-capillaries which run vertically to the plane of the section.

vessels on the other. Nowhere do blood-capillaries and bile-capillaries lie close beside one another; they are always separated by a gland-cell or by an intervening portion of the same. The most convincing demon-

stration of this is afforded by thin sections of rabbit's liver in which the blood-vessels have been transversely cut (Fig. 185); these also plainly show that the bile-capillaries run along the surfaces, the vascular capil-

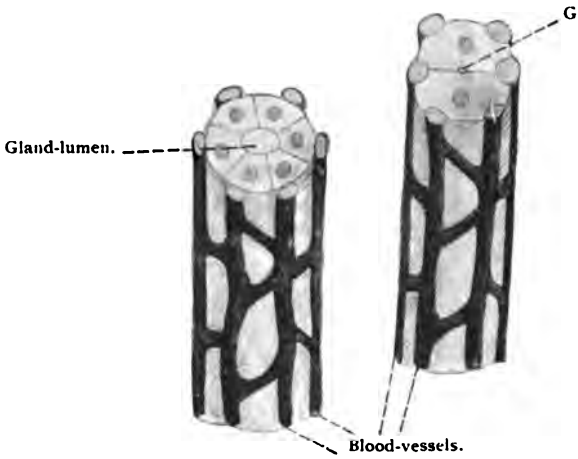



FIG. 184.—SCHEME OF AN ORDINARY GLAND-TUBULE (LEFT) AND OF A HEPATIC-TUBULE (RIGHT).

laries at the corners of the hepatic cells; however, this is not invariably the case, the bile-capillaries sometimes run along the edges, a behavior that occurs in particular in man (Fig. 185, X).

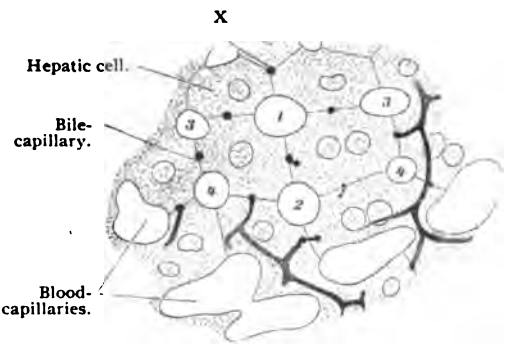



FIG. 185.—THIN SECTION OF THE LIVER OF A RABBIT, WITH INJECTED BILE-CAPILLARIES. × 560. (The drawing is *not* schematic.) Two of the hepatic cells are in contact with four blood-capillaries (1, 2, 3, 4). X. Bile-capillary at the edge of a hepatic cell.

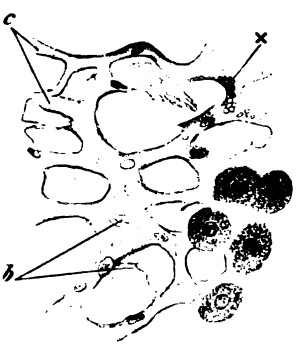



FIG. 186.—FROM A SHAKEN SECTION OF HUMAN LIVER. × 240. *c*, Blood-capillaries, at x still containing blood-corpuscles. *b*, Interlobular connective tissue. On the right are five hepatic cells; the others have fallen out of the meshes of the capillary network. Techn. No. 117.

The branches of the *hepatic artery* follow the course of those of the portal vein and ramify only in the interlobular tissue, where they form capillary networks about the larger bile-ducts and the branches of the

portal and the hepatic veins. The capillaries proceeding from the artery open into the portal interlobular veins or into the portal-capillaries at the margin of the lobules. In the capsule of the liver the hepatic artery forms a wide-meshed capillary plexus.

The course of the blood-vessels therefore is as follows: the portal vein enters at the transverse fissure, repeatedly divides into branches that steadily decrease in size and run in the connective tissue between the lobules as the interlobular veins. From these capillaries arise, which pass toward the axis of the lobule and terminate in the central vein. Several of the latter unite in the formation of each of the sublobular veins, which, like the larger hepatic veins they form by their union, run between the lobules.

The liver is provided with a capsule consisting of fibro-elastic tissue, capsula fibrosa (Glisson), which is especially well developed at the transverse fissure and in the form of special sheaths for the different channels * penetrates into the interior of the liver, where it is usually found in such small amount between the lobules that the boundaries of the latter are very imperfectly defined. Delicate fibers ("lattice fibers") derived from the interlobular connective tissue penetrate into the interior of the lobules, where for the most part they are arranged in the form of a delicate, radially-placed latticework.

The *lymph-vessels* accompany the branches of the portal vein, which netlike they embrace; with the portal-capillaries they enter the interior of the hepatic lobules, which, having arrived at the central vein, they then abandon. These *deep* lymphatics communicate with a superficial narrow-meshed network of lymph-vessels which occurs in the capsule of the liver.

The *nerves* chiefly consist of nonmedullated nerve-fibers, with which only a few medullated nerve-fibers are mingled; they enter the interior of the liver in company with the hepatic artery and follow its ramifications; their termination is unknown. Ganglion-cells occur along the course of the nerves.

The secretion of the liver, the *bile*, frequently contains drops of fat, also granular masses of bile-pigment. Columnar cells from the bile-ducts are to be regarded as incidental admixtures.

That the structure of the liver really follows the type of a tubular gland and that the cords of hepatic cells, with a few modifications, are comparable to the terminal compartments of other glands, the foregoing

* The walls of the veins are firmly attached to the liver substance by the interlobular connective tissue and for this reason they do not collapse when cut.

considerations have shown. The hepatic lobules, on the other hand, cannot without explanation be compared with the lobules of other glands; the latter as a rule consist of a tubular system, of which the excretory duct leaves the lobule at *one* place and continues into a larger excretory duct. In the hepatic lobules the excretory ducts emerge at *many* points on the surface of the lobule. The accompanying schematic representations may serve to elucidate the lobules. Imagine a tubular system; alongside the excretory duct runs an artery, the capillaries proceeding from it embrace the terminal compartments and open into a vein running along the base of the terminal pieces (Fig. 187). This is the principle of the behavior of each of the many tubular systems of which the liver consists; but there is one peculiarity: the somewhat tortuous

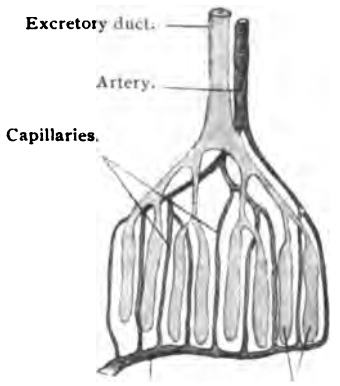



FIG. 187.—SCHEME OF A SYSTEM OF EXCRETORY CHANNELS ("TUBULAR SYSTEM").

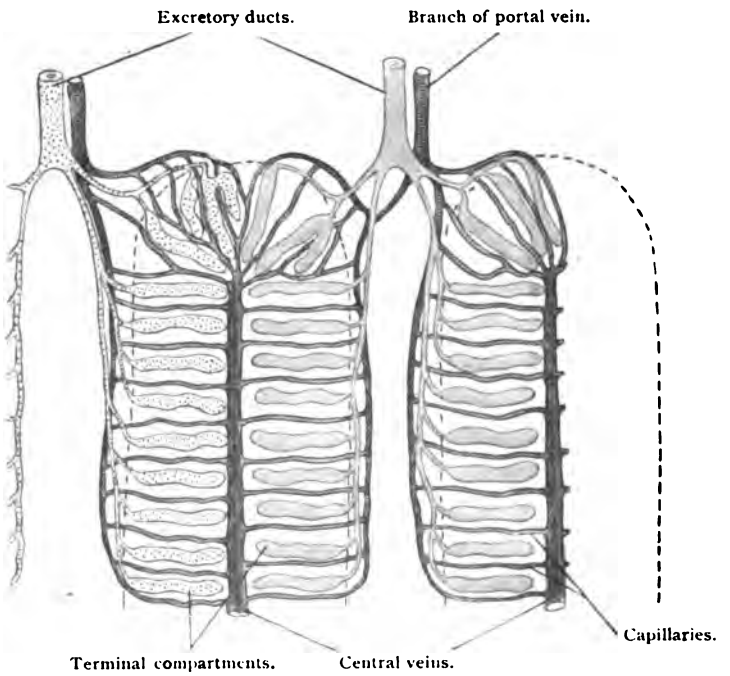



FIG. 188.—SCHEME OF THE LIVER. Two lobules are shown, of which the left is only half carried out. The ramifications and anastomoses of the capillaries and of the cords of hepatic cells have been omitted for the sake of clearness.

terminal compartments extend in different, definite directions (Fig. 188). At the base of the terminal piece, as well as above, runs the vein, but—another variation—the vein takes up not only these capillaries but also those of another side,* for there lies another tubular system, the terminal pieces of which at their base are in contact with the same vein. The vein, therefore, lies in the axis of a complex of terminal compartments and such a complex is termed an hepatic lobule. If now we draw a comparison with the scheme Fig. 187, the artery corresponds to the portal vein in scheme

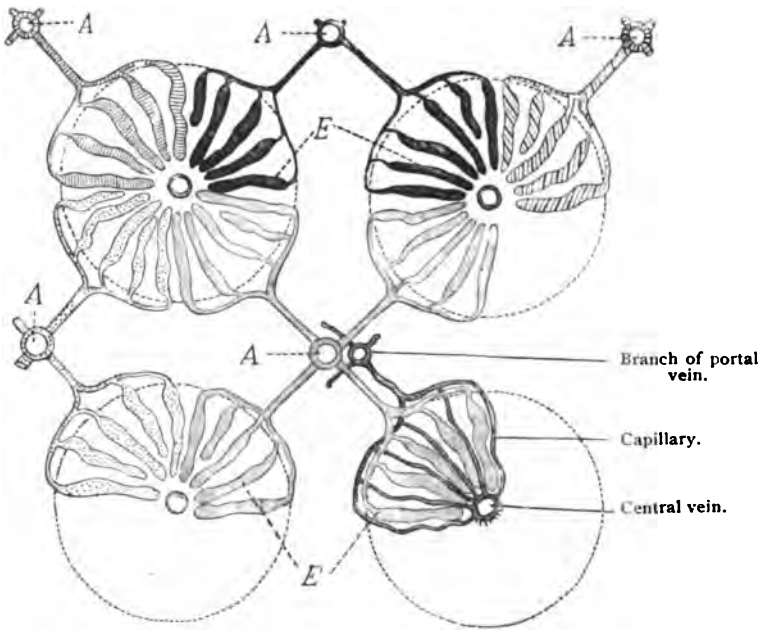



FIG. 189.—SCHEME OF A TRANSVERSE SECTION OF THE LIVER. Four lobules represented. The separate systems of ducts are indicated by the difference in shading. *A*. Excretory ducts. *E*. Terminal compartments.

Fig. 188, for the rôle of the portal vein in the liver is the same as that of arterial branches in other glands, and the vein in Fig. 187 is the equivalent of the central vein of Fig. 188; one hepatic lobule corresponds, not to one tubular system, but to parts of several systems (Fig. 189). For the sake of simplicity this schematic presentation is based on the conception of well-defined lobules, as they occur in the hog. In other animals the distribution of the terminal ramifications is less regular; the latter bend into neighboring lobules, to which in part is owing the less

* In the liver of the rabbit the central veins lie close under the surface and can take up the capillaries of one side only.

distinct demarcation of the lobules. Each tubular system participates in the formation of several lobules.

## THE PERITONEUM.

The peritoneum principally consists of bundles of fibrous connective tissue and numerous elastic networks; the free surface is covered by a simple layer of flat, polygonal epithelial- (endothelial) cells; the size of these cells varies according to the stretching to which they are subjected. The connection with subjacent parts (the parietes, the viscera, etc.) is effected by loose (subserous) connective tissue; in the peritoneum reflected over the small intestine the endothelial cells send delicate processes into the subserous tissue, that extend into the muscularis.

The *connective-tissue bundles* are arranged in thinner (in the visceral peritoneum) or thicker (in the parietal peritoneum, in the mesentery)

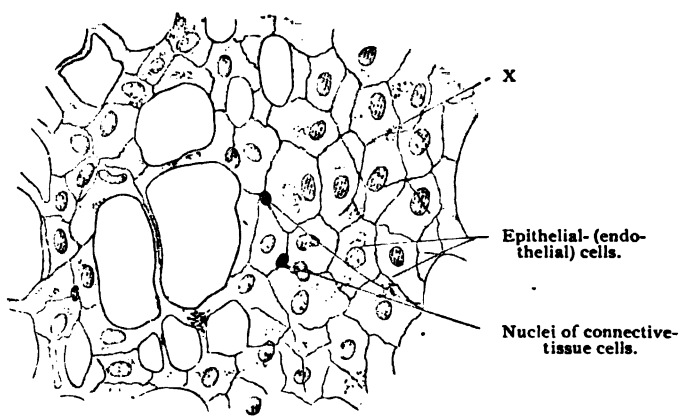



FIG. 190.—FROM THE GREATER OMENTUM OF A RABBIT. × 240. The network is formed by large and small bundles of connective tissue. The wavy striation of the bundles can only be indistinctly seen, because the preparation is mounted in damar.. At X the epithelial cells of the opposite surface can be seen shimmering through. Techn. No. 120.

layers, chiefly arranged parallel to the surface, and interlace in various directions; in certain localities (in the greater omentum, in the middle of the lesser omentum) the bundles form a beautiful network with polygonal or rectangular meshes. The strands of the network also are covered by flat epithelial-cells (Fig. 190).

The number of connective-tissue cells among the fibrous bundles is on the whole not large; only in young animals are larger groups of cells found; they resemble plasma-cells and all probably bear a close relation to the formation of blood-vessels.

The *elastic fibers* in the deeper layers of the peritoneum, particularly in the parietal portion, are profuse and vigorously developed.

The *subserous tissue* consists of loose connective tissue, many elastic fibers, and fat varying greatly in quantity; it is plentiful where the peritoneum is easily shifted over the underlying parts, but on the liver and the intestine so much reduced that it cannot be demonstrated as a special layer. At certain places, *e. g.*, in the broad ligaments, numerous bands of smooth muscle-fibers are found.

*Blood-vessels* and *nerves* are scantily represented; the latter partly terminate in lamellar corpuscles.

*Lymph-vessels* occur in the superficial and the deeper layers of the peritoneum.

## TECHNIC.

No. 90.—*Isolated Squamous Cells from the Oral Cavity.*—With a scalpel gently scrape the upper surface of the tongue and mix the scrapings on a slide with a drop of salt solution; apply a cover-glass; in addition to isolated, pale, squamous epithelial-cells, leucocytes ("salivary corpuscles") may be found; also, with more vigorous scraping, the tips of filiform papillæ, which not infrequently are surrounded by finely-granular, dark masses of micrococci to which tufts of leptothrix buccalis are attached. The preparation may be stained under the cover-glass with picrocarmine and then treated with dilute acidulated glycerol, provided too many air-bubbles do not make the preservation of the preparation impossible (Fig. 7, 1).

No. 91.—*Mucous Glands of the Lips.*—These are millet-sized nodules perceptible to touch and accessible for macroscopic preparations. For microscopic preparations cut from the mucous membrane of a human lower lip (not the margin of the lip) 1 cm. cubes; fix them in 50 c.c. of Kleinenberg's picrosulphuric acid and in twenty-four hours harden in 50 c.c. of gradually-strengthened alcohol. In three days the tissue may be sectioned. Cut many sections, not too thin, and stain them with hematoxylin; place the sections in water and with the naked eye select those which include the excretory duct and preserve them in damar; examine with a low power (Fig. 134).

No. 92.—*Dried Ground Tooth.*—To prepare dried ground sections of teeth they should be obtained immediately after they are extracted, sawed into transverse discs 2 mm. thick, glued with sealing-wax upon cork and treated like No. 56. If longitudinal sections are desired the entire tooth should be glued to the cork. Longitudinal sections are to be preferred, since they show all parts of the tooth in a single preparation. If it is desired to decalcify the teeth of an adult, proceed as with No. 58. The enamel consisting of earthy salts and only from 3 to 5 per cent. of organic substances dissolves completely, so that only the dentine and cementum remain (Fig. 135, 136, 137).

No. 93.—*Odontoblasts.*—Remove the teeth from the jaws of a newborn child; place them in 60 c.c. of Müller's fluid; after six days the pulp can be easily withdrawn in toto by means of forceps. With the scissors cut from the upper surface of the pulp a piece the size of a lentil and tease a little the tolerably tenacious tissue in a drop of Müller's fluid; apply a cover-glass, press lightly upon it, and examine with the high power. At the edges of the preparation the long processes of the odontoblasts standing out like hairs will be seen; also scattered, completely isolated odontoblasts (Fig. 139). In order to preserve, treat under the cover-glass with distilled water for two minutes, then with picrocarmine; when the staining is completed, add dilute acidulated glycerol.

No. 94.—*Enamel Prisms.*—These are obtained by teasing portions of the lateral surface of the teeth of No. 93 in a drop of Müller's fluid. Examine with a high power. The enamel prisms will be found in groups of three and more; they are distinguished by their dark outlines and usually indistinct cross-striation (Fig. 138). Mount in glycerol. The prismatic form of the enamel prisms may be seen in thin sections cut parallel to the surface of the teeth. Only portions of a section exhibit regular hexagonal prisms, that is, cross-sections of the prisms (Fig. 138). The enamel of young teeth may be sectioned without previous decalcification.

No. 95.—*Development of Teeth.*—For the study of the early stages select pig and sheep embryos; these are the most easily obtained at the slaughter houses; for the first stage the pig embryos should have a size of about 6 cm., for the second stage a size of about 10 or 11 cm. For later stages the inferior maxilla of newborn dogs or cats is very suitable. Place the heads (or the lower jaws) in 100 c.c. of Kleinenberg's picrosulphuric acid for from twelve to twenty-four hours and harden in from 80 to 120 c.c. of gradually-strengthened alcohol. After the heads have lain six or eight days in 90 per cent. alcohol, they are to be decalcified in 100 c.c. of distilled water plus 1 or 2 c.c. of nitric acid. When the decalcification is completed, in from three to eight days, harden again in alcohol. In five or six days cut off the lower jaw and divide it in front in the middle (larger jaws should be cut vertically into pieces 1 or 2 cm. long); stain the pieces in bulk in borax-carmine. When the staining and decolorization are completed, the tissue is to be transferred to absolute alcohol, in which it must remain for several days; it is then to be embedded in liver and sectioned. It is necessary to cut many (20 to 40) thick sections, since only those which pass through the middle of the tooth, or the anlage of the tooth, can be used. Mount in damar. Not infrequently in sectioning the enamel organ is lifted from the papilla, so that a free space exists between the two. The dentine is often stained in different tones of red; this is due to the different ages of the calcified and uncalcified strata of the dentine. The objects may also be fixed in Müller's or in Zenker's fluid; section-staining in hematoxylin is not advisable, since too many sections must be stained which on investigation are found to be useless.

No. 96.—*Papillæ Filiformes, Fungiformes, Circumvallatæ; Follicles of the Tongue.*—Cut pieces 2 cm. square from the mucous membrane of the surface of a human tongue. Each piece should have some of the muscle tissue attached to its lower surface; for fungiform papillæ cut the piece from the tip of the tongue; for filiform, from the middle of the dorsum of the tongue; for circumvallate, from the root of the tongue, and for follicles (the punctiform openings of which can be seen with the naked eye) from the root of the tongue, and place them in 100 or 200 c.c. of Müller's fluid. The fluid must be changed several times; after two weeks wash the tissue and harden it in 50 c.c. of gradually-strengthened alcohol. For filiform papillæ cut thick saggital sections of the tongue and do not stain them; stain the other sections in Hansen's hematoxylin and mount in damar (Fig. 145, 146, 147). For the preparations represented in Fig. 148 and Fig. 151 the tissue was fixed and hardened in 50 c.c. of absolute alcohol. Rabbits' tongues may be placed in toto in 200 c.c. of Müller's fluid; the subsequent treatment is the same. Thick cross-sections through the anterior half of the entire tongue are suitable for the study of the arrangement of the muscles of the tongue. Thin sections of the root of the tongue show beautiful mucous and serous glands.

No. 97.—*The Tonsils.*—The tonsils of adult man do not furnish instructive preparations. They should be treated according to Téchn. No. 96. The tonsils of the rabbit and the cat are recommended; to find them proceed as follows.

Dissect the skin from the anterior surface of the neck and remove the structures lying over the trachea and esophagus; with a pair of stout scissors cut through both tubes above the sternum, grasp the cut ends with forceps and with scissors dissect them up to the head of the pharynx, keeping close to the anterior surface of the vertebral column (at the same time the cornua of the hyoid bone will be divided). Cut through the musculature close to the median edges of the inferior maxilla, also through the ligaments of the tongue (glosso-epiglottic). (In the rabbit it is advisable to divide both angles of the mouth, and with scissors introduced within the slit to sever the ligaments and the genio-hyoglossus muscle.) Draw the trachea and attached structures downward, press the tongue down between the rami of the inferior maxilla, and divide its remaining attachments (to the palate) close to the bone. Put the tongue down with its free surface looking upward. With delicate scissors divide the posterior wall of the pharynx in the median line down to the larynx and pull the walls apart; the tonsils will then be seen as a pair of oval prominences, about 5 mm. long, on the lateral walls of the pharynx. They may be fixed in 60 c.c. of Kleinenberg's picrosulphuric acid (p. 21), and hardened in 50 c.c. of gradually-strengthened alcohols (p. 33), stained with hematoxylin or with eosin and hematoxylin (p. 37), and mounted in damar.

No. 98.—*The Esophagus.*—Pieces of human esophagus 2 cm. square and of that of the rabbit and cat 2 cm. long of the entire tube are to be

fixed in 60 c.c. of Müller's fluid and in two weeks hardened in 50 c.c. of gradually-strengthened alcohol; stain with Hansen's hematoxylin; mount in damar (Fig. 152).

No. 99.—*The Mucous Membrane of the Stomach.*—For topographical preparations place pieces from 2 to 5 cm. square for six hours in 100 c.c. of 3 per cent. nitric acid. Remove the gastric contents adhering to the mucous membrane by moving it slowly to and fro in the acid. In a half hour renew the acid; harden in 60 c.c. of gradually-strengthened alcohol. Mount thick unstained sections in damar (Fig. 153).

No. 100.—*Fresh Gastric Glands.*—From the fundus of the stomach of a rabbit just killed cut pieces about 2 cm. square and separate the loosely-attached muscular coat from the mucous membrane. Grasp the latter with forceps at the left edge and with fine scissors cut very thin strips, 0.5 to 1 mm. thick; tease them in a drop of 0.5 per cent. salt solution. The body and fundus of the fundus glands can be satisfactorily isolated without much trouble. The protoplasm of theparietal-cells can be distinctly seen (Fig. 191, *B*), the chief-cells are invisible. The nuclei may be stained with picrocarmine and the preparation mounted in dilute glycerol. The isolation of the pylorus glands can only be accomplished by very careful teasing.

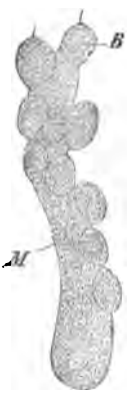



FIG. 191.—LOWER HALF OF AN ISOLATED FUNDUS-GLAND OF A RABBIT. × 240. *B*, Parietal-cell; *M*, membrana propria.

No. 101.—*Isolated Gastric Epithelium.*—Place pieces 1 cm. square of gastric mucous membrane for about five hours in 30 c.c. of Ranvier's alcohol (see further p. 29). In the majority of the cells the mucous portion occupies a large division and they have the appearance of those pictured in Fig. 15, *c*. The preparation may be stained under the cover-glass with picrocarmine, and mounted in diluted acidulated glycerol.

No. 102.—*Gastric Glands.*—The stomach of a cat or dog that if possible has been fasting for one or two days is especially recommended. The stomach of the rabbit, on account of the very small size of the chief-cells, is less suitable. Dissect off the mucous membrane from the muscular coat and place pieces of the former about 1 cm. square in about 10 c.c. of absolute alcohol. In about a half-hour transfer them to 20 c.c. of fresh alcohol. The outlines of the glands can be recognized in moderately thin sections; the only difficulty is the circumstance that the gland-tubules are placed very close together. The beginner may not recognize the glands and may mistake for them the gastric pits lined with clear epithelium. The stomach of man, which however is suitable for use only for a few hours after death, exhibits this difficulty in a less degree. For the study of the minute structure of the glands and of the superficial epithelium, embed the tissue in liver and cut the thinnest possible sections.

*a. For fundus glands, chief- and parietal-cells*, cut vertical or, better, horizontal sections of the mucous membrane and stain them with Hansen's hematoxylin for two or four minutes. Wash the sections thoroughly in 30 c.c. of distilled water, which must be changed as often as it becomes bluish—about once or twice. Transfer them to 5 c.c. of a $\frac{1}{30}$ per cent. solution of Congo red (p. 25), for from three to six minutes, wash two minutes in distilled water and mount in damar. If the sections are too thick, everything appears red; the large red parietal-cells cover the smaller chief-cells; examine the thinnest parts of the sections, especially the fundi of the glands, where the parietal cells are not so exceedingly profuse. The parietal cells can be recognized with the low power as isolated red spots on a rose-red ground. With the high power the pale blue smaller chief-cells can be seen. The very narrow lumen of the fundus glands may be best seen in cross-sections (sections parallel to the surface of the mucosa). The lateral twigs of the chief lumen can only be perceived in very favorable sections (Fig. 155). Fig. 154 is a combination of several thin longitudinal sections.

*b. For pylorus glands*, stain vertical and horizontal sections of the mucosa with Hansen's hematoxylin and mount in damar. The lumen of the pyloric glands is wider (Fig. 157). Owing to the extreme sinuosity of the glands, thin sections contain but few glands cut in their entire length, mostly only parts of them.

No. 103.—*Duodenal Glands.*—Cut out the stomach and duodenum of a cat about one hour after death. Open both along their length, remove the contents by swaying them gently to and fro in salt solution (p. 19), and place the pyloric end of the stomach and the upper half of the duodenum, in all a piece 5 or 6 cm. long, for six hours in 100 c.c. of 3 per cent. nitric acid. Further treatment like No. 99. Cut longitudinal sections, which simultaneously pass through pylorus and duodenum. Stain with Hansen's hematoxylin. Mount in glycerol or in damar (Fig. 163). If the tissue be placed in the acid immediately after death the smooth muscle of the intestine contracts so that a rigid curving of the intestinal wall takes place.

No. 104.—*Epithelium and Villi of the Small Intestine.*—From the middle portion of the small intestine of a rabbit just killed, cut a piece one cm. long, open it along its length and remove the contents by carefully pouring over it ¾ per cent. salt solution. Then grasp the piece at the left edge with the forceps, with fine scissors cut off a small strip and spread it out in a drop of salt solution on a slide on a black background. With the unaided eye one can see the villi projecting from the edge of the preparation. Examine the preparation without a cover-glass, with the low power. The villi will be seen partly extended, partly contracted; the latter condition may be recognized by transverse folds running across the villi (Fig. 192). Details cannot be

FIG. 192.—INTESTINAL VILLUS OF A RABBIT. × 70.

detected. Apply a cover-glass; the villi thus become flattened and appear clearer; the cylindrical epithelium and close beneath this the loops of the capillary blood-vessels can be distinctly seen. If the epithelium contains goblet-cells, these appear as bright shining rounded spots. For the investigation of the epithelium, proceed as follows:—

*a.* Tease the piece a little; in this way columnar cells, singly and in groups, may be isolated, which are to be examined with the high power. Not infrequently some columnar cells are found inflated and of a spherical form. The basal border sometimes shows very distinct rods. Goblet-cells, when present, may be recognized by their homogeneous appearance and if carefully focused the sharply-outlined orifice may be perceived. Occasionally the epithelial-cells are difficult to loosen from the basement-membrane; in such cases make a second investigation an hour later, when the epithelium will be sufficiently macerated to be brushed off.

*b.* For permanent preparations place pieces (1 cm. square) of the intestine in 30 c.c. of Müller's fluid. In three or five days take the tissue out, scrape it with the tip of a scalpel, and distribute a little of the scraping in a drop of diluted glycerol; cover-glass; high power (Fig. 161, A).

No. 105.—*Sections of the Small Intestine.*—Place pieces from 2 to 4 cm. long of the intestine of a rabbit, better, of a puppy or a kitten, in 100 or 200 c.c. of 3 per cent. nitric acid. After six hours the pieces are to be hardened in 100 c.c. of gradually-strengthened alcohol. Sections can be made through the entire intestinal tube; in most cases, only fragments of the villi are thus obtained; to obtain entire villi, with a razor cut open the hardened intestine along its length, pin it with needles on a cork-plate, with the mucosa uppermost. The villi can then be seen with the unaided eye. Cut thick cross-sections, stain them for one minute with Hansen's hematoxylin and mount in damar. Goblet-cells are very frequently found in the epithelium (Fig. 161, B). Staining in bulk with borax-carmine is highly recommended.

The human intestine, before being placed in the nitric acid, must be cut open and washed in the same fluid. It is advisable to pin pieces about 5 cm. square to a cork-plate and thus to place them in the fixing and hardening fluids. If the intestine is not absolutely fresh, the entire superficial epithelium loosens so that the naked connective-tissue villi lie exposed.

Horizontal sections of the intestine furnish very beautiful pictures. Not infrequently the cross-sections of the glands drop out and then only the connective-tissue tunica propria remains. In these preparations the goblet-cells all appear as clear bodies of equal size and therefore afford no clue in regard to the functional state of the cell.

For the latter purpose the following is recommended:—

No. 106.—*Triple Staining of the Intestine.*—Small pieces of tissue are to be fixed in Flemming's mixture (p. 22), hardened in gradually-strengthened alcohol, and subsequently treated according to the method given on page 40, 10.

No. 107.—*Agminated Nodules* (*Patches of Peyer*).—These can be seen shimmering through the uninjured fresh intestinal wall of the rabbit, but in the dog and in the cat (on account of the thickness of the muscular coat) often they are not perceptible. In the latter animals patches are constant at the point where the small intestine opens into the large. Cut out the portion of the intestine of a rabbit containing the Peyer's patches and proceed according to the method given in No. 105. In the cat take the lowermost portion of the ileum (about 2 cm. long) with a piece of the cecum of the same length; open both along their length and span them out on a cork-plate, with the mucosa uppermost. Usually the mucosa is covered with a tenaceous excrement, difficult to remove by washing, which glues the villi together, so that only oblique sections of the villi can be obtained. Further treatment like No. 105.

Closely-placed nodules are found in the blind half of the vermiform process of the rabbit, which encroach upon the mucosa and compress it to such narrow areas that cross-sections exhibit very complicated pictures, scarcely intelligible to the beginner.

Fixation in 0.1 per cent. chromic acid and hardening in gradually-strengthened alcohols renders the germinal centers very distinct, but is not so good for the remaining elements as the nitric acid.

No. 108.—*The Large Intestine.*—Treat empty pieces like No. 105 or No. 106 (compare with Fig. 16, p. 70). Pieces filled with feces must be cut open, washed, and spanned on cork.

No. 109.—*Fresh Crypts of the Large Intestine of the Rabbit.*—Cut a piece 1 cm. long from the lowermost portion of the large intestine (between two spherical masses of feces), place it on a dry slide, open it with the scissors and spread it out with the mucous surface uppermost; add a drop of ¾ per cent. salt solution, grasp the piece with forceps at the left edge and with fine scissors cut off an extremely thin strip. Transfer this with a drop of the salt solution to another slide; with needles separate the muscularis from the mucosa and tease the latter a very little; apply a cover-glass with slight pressure. With a low power the crypts can be readily seen, but it is difficult to detect their orifices (Fig. 193). The epithelial-cells are often granular in the portion bordering the lumen. With the high power the superficial epithelium can be very well seen from the side and from the surface. The contents of the goblet-cells often are not clear, as in sections, but dark and granular.

FIG. 193.—*e*, Epithelium; *l*, crypts. ×80.

No. 110.—*Blood-vessels of the Stomach and the Intestines.*—A stomach and intestine injected from the descending aorta are to be fixed in from 50 to 200 c.c. of Müller's fluid and hardened in gradually-strengthened alcohols. One portion should be cut into thick (up to 1 mm.) sections, stained, and mounted in damar (Fig. 166), and another part used

for horizontal preparations, which with the low power and change of focus are very instructive. For this purpose pieces of the large intestine 1 cm. square may be transferred from absolute alcohol to 5 c.c. of turpentine for clearing and mounted in damar. It is also easy to strip the muscularis from the mucosa and to mount the separate coats in damar.

No. 111.—*Auerbach's and Meissner's Plexus.*—For this purpose intestines with a thin muscular coat are preferable, therefore the intestine of the rabbit and guinea-pig (not of the cat) are especially suitable. It is not necessary that the object be absolutely fresh; the small intestines of children several days after death can still be used. Prepare 200 c.c. of a dilute solution of acetic acid (10 drops of glacial acetic acid to 200 c.c. of distilled water). Then separate a piece (from 10 to 30 cm. long) of the small intestine from the mesentery. Cut it off and with the finger lightly press out the contents; tie the lower end of the intestine and fill it from the upper end with the dilute acetic acid; tie it above and place the whole piece in the remainder of the acetic acid. In one hour change the fluid. In twenty-four hours transfer the intestine to distilled water, with scissors open it along one side of the line of attachment of the mesentery and cut off a piece 1 cm. long. The muscularis can be readily separated from the mucosa with the aid of forceps; they are only firmly united at attachment of the mesentery.

*a. Plexus Myentericus.*—If a piece of black paper be placed under the glass dish containing the tissue, the white nodal points of Auerbach's plexus can be seen by the unaided eye. Transfer a piece of the muscularis, about 1 cm. square, in a drop of the dilute acetic acid to a slide; examined with the lower power it furnishes a very pretty picture (Fig. 167, A). If it is desired to preserve thepreparation, place the tissue for one hour in 30 c.c. of distilled water, which must be changed several times, and then for from eight to sixteen hours in 5 or 10 c.c. of a 1 per cent. osmic acid solution, *in the dark;* wash the piece quickly in distilled water and mount in diluted glycerol. The osmium preparations are not as beautiful as the fresh ones in the acetic acid. In the guinea-pig both strata of the muscularis can be readily separated (if the intestine is absolutely fresh on being filled with the dilute acid); the plexus remains attached to one stratum. Pieces of this should be placed for one hour in distilled water, then treated with gold chlorid (p. 43), and mounted in damar. The gold-chlorid treatment is less adapted to human intestines, since both the muscular layers are likewise stained red and partially conceal the plexus. The firm union of the muscular strata in the human organ may be due to the age of the object.

*b. Plexus Submucosus.*—With a scalpel scrape the epithelium from the isolated mucosa; place a piece about 1 cm. square on a slide; apply a cover-glass, press upon it slightly, and examine with the low power (Fig. 167, B). To preserve the preparation, proceed as in No. 111, *a;* but it is advisable to span the pieces on cork and before transferring them from the ninety-five per cent. alcohol to the bergamot oil, to press them

somewhat, in order that the alcohol may be completely removed from the spongy mucosa.

In addition to nerves many blood-vessels are present, which may be easily recognized by the structure of their walls, in part by the transversely-placed nuclei of the muscle-fibers.

No. 112.—*The Parotid, Submaxillary, and Sublingual Glands.*—From human glands (in winter useful after three or four days) cut a number of pieces from 0.5 to 1 cm. square and place them in 30 c.c. of Zenker's fluid (for further treatment, see p. 31). Stain one piece in bulk in borax-carmine. Embed another in liver and cut the thinnest possible sections; small fragments about 2 mm. long can be used; stain them in Hansen's hematoxylin, two or three minutes; the transfer of the sections to the staining solution must be done slowly, or the most delicate sections will be destroyed; then stain with eosin (p. 37), and mount in damar. (Very thin sections should be examined in water after the staining in hematoxylin is completed, since the cell boundaries are then very much more distinct). If the staining is successful, the salivary tubules and the crescents are red. In the sublingual gland and in the mucous cells of the submaxillary the membrana propria also stains red; it must not be confused with the sections of the crescents, which latter are granular, while the membrana propria has a homogeneous appearance. The mucous cells in the borax-carmine preparations are clear throughout. In the sections stained with hematoxylin they are sometimes clear, sometimes a pale blue of different shades (Fig. 168); the portion which stains is a reticulum which occurs in certain functional stages of each mucous cell. The very short intercalated pieces of the submaxillary gland are difficult to find; on the other hand, they may be easily seen in the parotid (Fig. 169, *s*) (also in that of the rabbit). Of the end-pieces only certain portions, those which have been accurately halved and the lumen of which is visible, are suitable for study. The numerous oblique and tangential sections are often very difficult to understand (Fig. 168, 4, 5, 6, 7).

No. 113.—*The Pancreas.*—The human pancreas as a rule cannot be used. The treatment is the same as for the parotid gland, No. 112. The characteristic granular zone of the gland-cells bordering the lumen is not to be seen by this method (Fig. 171, B). Tease a pinhead-sized piece of the fresh pancreas of a cat in a drop of ¾ per cent. salt solution. With the low power the end-pieces appear spotted; this is due to the partly clear and partly granular divisions of the cell. With high magnification the tissue appears like Fig. 171, A.

No. 114.—*Liver Cells.*—Make an incision in a fresh liver and with the blade of a scalpel obliquely placed scrape the cut surface. The brown liver-tissue adhering to the blade is to be transferred to a slide and a drop of salt solution added. Apply a cover-glass. Examine first with the low power then with the high (Fig. 176, A). In addition to the liver-cells, the preparation contains numerous colored and colorless blood-corpuscles.

No. 115.—*Hepatic Lobules.*—Place small pieces (about 2 cm. cubes) of a pig's liver in from 30 to 50 c.c. of absolute alcohol. The majority of the lobules are hexagonal; they can be seen on the surface of the liver by the unaided eye and after a moment become distinctly visible on the cut surface. The section of the central vein also becomes visible. In about three days sections can be made; stain them with Hansen's hematoxylin. The division into lobules can be well seen with the low power, but the hepatic cells as well as the bile-ducts are less satisfactory for study. Better for this purpose is the following.

No. 116.—*Human Liver.*—Place pieces about 2 cm. square, as fresh as possible, for four weeks in 200 c.c. of Müller's fluid for fixation and then in 100 c.c. of gradually-strengthened alcohols for hardening. Examine unstained sections (cut parallel and also vertical to the surface) and stain others with Hansen's hematoxylin and eosin; mount in damar. The demarcation of the lobules is not distinct, because of the slight development of the interlobular connective tissue. The division into lobules may be more readily perceived on macroscopic inspection, than on investigation with the microscope. For orientation the beginner should recall that isolated sections of blood-vessels always represent intralobular veins; while groups of such sections represent branches of the portal vein, of the hepatic artery, and of the bile-duct. Exact transverse sections of central veins may also be recognized by the cords of hepatic cells radiating from them (Fig. 177). For the study of the structure of the gall-bladder as well as of the larger bile-ducts, only absolutely fresh livers can be used, since the alkaline bile permeates the walls of the gall-bladder soon after death, stains the tissue yellow, and renders it unfit for microscopic investigation.

No. 117.—To demonstrate the *capillaries* and the *intralobular connective tissue*, which in ordinary preparations are scarcely visible, shake a number of thin double-stained sections of human liver (No. 116) for from two to three minutes in a test-tube half filled with distilled water. The liver-cells in part fall out; the edges of the preparation are then to be examined in a drop of water (Fig. 186). This preparation can be mounted in damar, but the more delicate connective-tissue fibers disappear therein.

No. 118.—*Blood-Vessels of the Liver.*

*a.* Chloroform a rabbit and quickly place a 2 cm. cube of liver (without allowing much blood to flow from it) in 50 c.c. of absolute alcohol. In two days the natural injection can be seen on the surface; it is indicated by brown spots within the centers of the lobules. Cut thick sections parallel to the surface and mount them unstained in damar. Examine with a low power. Very frequently only the superficial strata of the liver contain filled blood-vessels.

*b.* Of all injections that of the liver is most easily accomplished. Inject Berlin blue (p. 43), either through the portal vein or the inferior vena cava; in the latter case it is advisable to make an incision above

the diaphragm, to allow the heart to rest upon the latter, and to insert the canula through the right auricle into the inferior cava. The injected liver is to be placed in toto in about 500 c.c. of Müller's fluid; after six days pieces about 2 cm. square of the portions best injected are to be cut out, again placed for two or three weeks in about 150 c.c. of Müller's fluid, and finally hardened in 100 c.c. of gradually-strengthened alcohols. Cut thick sections and mount them unstained in damar (Fig. 180, 181, 182).

No. 119.—*Exhibition of Gland Lumina by Golgi's "Black Reaction."* —Place small pieces of the root of the tongue, of the stomach, of the salivary glands, and of the liver for three days in the osmio-bichromate mixture (in winter, in the warm chamber), and for the same length of time in the silver solution. For further treatment see page 41. Very often the staining does not succeed until after the procedure has been repeated one or twice. After-staining (p. 42) is advised. In the liver the "lattice-fibers" occasionally stain.

No. 120.—*The Endothelium of the Peritoneum.*—Proceed as in No. 38, but instead of taking the mesentery, which also yields instructive pictures, use the greater omentum. The pieces may be stained in Hansen's hematoxylin and mounted in damar (Fig. 190).

# VII. THE RESPIRATORY ORGANS.

## THE LARYNX.

The *mucous membrane* of the larynx is a continuation of the pharyngeal mucous membrane and like this is composed of an epithelium, a tunica propria, and a submucosa, which latter connects the mucous membrane with underlying parts. The mucous membrane over nearly the whole of the organ is covered by a stratified ciliated columnar *epithelium;* the ciliary wave is directed toward the cavity of the pharynx. On the true vocal cords, on the anterior surface of the arytenoid cartilages and on the laryngeal surface of the epiglottis the epithelium is of the stratified scaly variety. The *tunica propria* consists of numerous elastic fibers and of fibrous connective tissue, which in the lower animals is condensed to a membrana propria immediately beneath the epithelium. The tunica propria is the site of a varying number of leucocytes; in dogs and cats, solitary nodules (p. 125) are found in the mucous membrane of the ventricle of the larynx (Morgagni). Papillæ mainly occur in the mucous membrane clothed with stratified squamous epithelium; on the free border and on the lower surface of the vocal cords the papillæ are merged in longitudinal ridges. The submucosa contains branched tubular mucous glands from 0.2 to 1 mm. in size.

The cartilages of the larynx principally consist of the hyaline variety, which in a measure exhibits the peculiarities of the costal cartilage. The hyaline cartilages are the thyroid, the cricoid, the greater portion of the arytenoids, and often the triticeous cartilages. The epiglottis, the cuneiform cartilages (Wrisberg), the cornicular cartilages (Santorini), the median portion of the thyroid, and the apex and vocal process of the arytenoid cartilages are of the yellow elastic variety. Occasionally the triticeous cartilages are composed of fibro-cartilage. Between the twentieth and thirtieth years of life ossification (chiefly endochondral) begins in the thyroid and the cricoid cartilages.

The larynx is richly supplied with *blood-vessels* and *nerves*. The blood-vessels form two or three networks extending in planes parallel to the surface and a close subepithelial capillary plexus.

The *lymph-vessels* form two communicating networks also extending

in horizontal planes, of which the superficial consists of narrower channels and lies beneath the vascular capillary network.

The *nerves* in their course include microscopic ganglia. In part they terminate in end-bulbs and in taste-buds.

## THE TRACHEA.

The ciliated mucous membrane of the trachea possesses a structure like that in the larynx, excepting only that the elastic fibers form a close network in which the fibers pursuing a longitudinal direction predominate. This network lies immediately beneath the epithelium, above the glands. The cartilages are of the hyaline variety. The posterior wall of the trachea is composed of a layer of transversely-arranged smooth muscle-fibers, that usually is covered by a stratum of muscle-fibers extending longitudinally. The mucous glands of the posterior wall are distinguished by their size (2 mm.); they not infrequently penetrate the muscular layer, so that they lie in part in the fibrous tissue behind it.

The behavior of the blood-vessels, lymph-vessels, and nerves is the same as in the larynx.

## THE BRONCHI AND THE LUNGS.

The lungs may be regarded as compound alveolar glands, in which, as in all glands, excretory and secretory (in this case respiratory) portions may be distinguished. The *excretory division* comprises the larynx, the trachea, and the bronchi. Each bronchus on entering the lung divides repeatedly and within the same undergoes continual subdivision, by direct giving off small lateral twigs, by branching at acute angles, and by gradual decrease in the caliber of the large branches; in this way each bronchus breaks up into minute twigs, that nowhere anastomose with one another and that retain the characteristics of the excretory duct to a diameter of 0.5 mm.

At this point the *respiratory division* begins. Small, isolated, hemispherical evaginations, *the alveoli*, appear at irregular intervals on the walls of the minute bronchi. Such bronchi are called *respiratory* or *terminal bronchioles*. These divide and lead into the *alveolar ducts*, which differ from the bronchioles only in the larger number of alveoli in their walls. The alveolar ducts divide at right or acute angles and pass without sharp demarcation into the slightly-expanded blind *terminal vesicles* (less correctly, infundibula), the walls of which are thickly beset with alveoli. Each alveolus is open, not only toward the terminal vesicle,—

this broad opening is termed *base*—but also is in direct communication with neighboring alveoli by means of minute canals, the so-called *pores*.

The entire respiratory division is separated by areolar tissue into *lobules* from 0.3 to 3 cm. in size. All the branches of the excretory division to a diameter of from 1.5 to 1 mm. and less lie between the lobules, as "interlobular ducts."

The *minute structure of the bronchi* in the largest branches does not

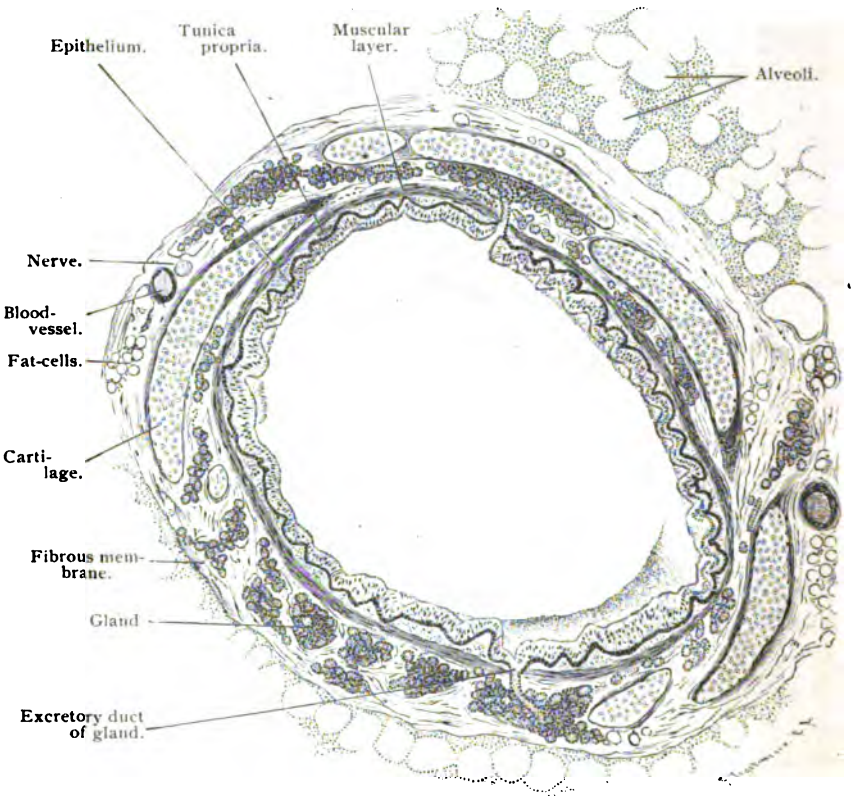



FIG. 194.—CROSS-SECTION OF A BRONCHUS, TWO MILLIMETERS THICK, OF A CHILD. × 30. Techn. No. 123.

differ from that of the trachea. Gradually modifications appear, which first involve the cartilages and the musculature. The C-shaped *ring cartilages* are replaced by irregular plates lying on all sides of the bronchial wall. They diminish in size and thickness with the decrease in the diameter of the bronchi and disappear in bronchioles 1 mm. in diameter.

The *smooth muscle-fibers* are circularly disposed in a continuous layer lying within the cartilages and form a complete investment for the

tube. The thickness of the muscular layer decreases with the diameter of the bronchi; but muscle-fibers are still found as far as the alveolar ducts. They are wanting in the terminal vesicles.

The *mucous membrane* is thrown into longitudinal folds and consists of a stratified ciliated epithelium containing goblet-cells, that in the smaller bronchi becomes gradually reduced to a single stratum, and of a connective-tissue tunica propria. The latter contains numerous longitudinal networks of elastic fibers and leucocytes in greatly varying numbers. Occasionally the latter form solitary nodules, from the crest of which leucocytes wander through the epithelium into the bronchial tube.

Branched tubular *mucous glands* occur as far as the cartilages extend; they are situated outside of the muscular layer (Fig. 194). They are numerous and do not disappear until at the beginning of the respiratory bronchioles.

External to the cartilages is a *fibro-elastic tunic*, which envelops the entire bronchus including the accompanying vessels and nerves.

The *minute structure of the respiratory division*, after the gradual disappearance of cartilages and glands, is distinguished in particular by the nature of the epithelium.

The *respiratory bronchioles* following the smallest excretory bronchi

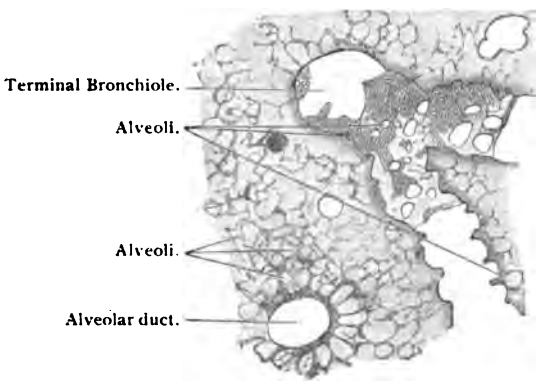



FIG. 195.—FROM A SECTION OF LUNG OF ADULT MAN. × 50. The terminal bronchiole divides into two branches (on the right). A portion of the wall of the bronchiole fell within the plane of the section; here the entrance to the alveoli is seen from above; in the lower branch the alveoli are viewed from the side. The epithelium of the bronchiole is mixed. The epithelial lining of the alveoli is only partially visible with this magnification. Techn. No. 124.

at the beginning still contain a single layer of ciliated columnar epithelium; as they proceed the cilia are lost, the cells become cubical, and between these another kind of epithelial-cells appears, in the form of thin nonnucleated plates of different sizes. These plates and isolated or small groups of cubical cells form an epithelium called *respiratory*

*epithelium.* The transition of the cubical epithelium into the respiratory epithelium is not abrupt, but occurs in such wise that at one extremity of the bronchiole cubical, at the other extremity respiratory epithelium is found, or that groups of cubical cells are surrounded by respiratory epithelium and the reverse. Hence the respiratory bronchioles contain a mixed epithelium (Fig. 195 and Fig. 196, *A*).

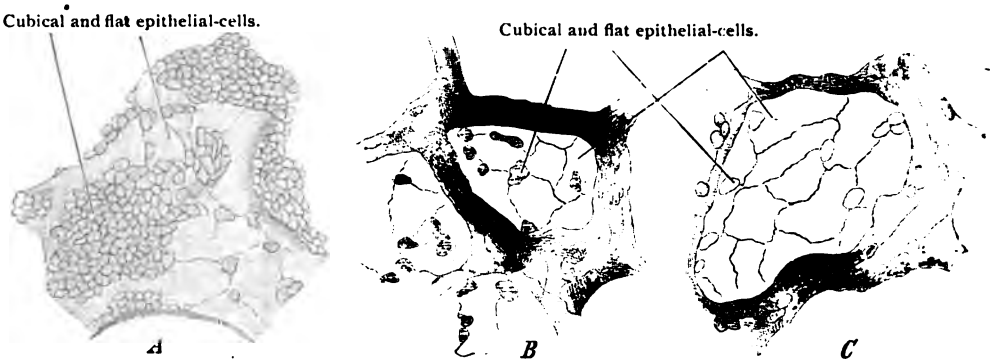



FIG. 196.—FROM SECTIONS OF HUMAN LUNG (A AND B), AND (C), OF LUNG OF A KITTEN NINE DAYS OLD. × 240. *A*. Mixed epithelium of terminal bronchiole. *B* and *C*. Alveoli drawn with change of focus. The margin of the alveolus is shaded; it can be seen that the epithelium covering it is like that in the depth of the alveolus (the light portion); the nuclei of the cells are not visible. Techn. No. 124.

The epithelium of the *alveolar ducts* and of the *alveoli* is the same as the respiratory epithelium of the bronchioles. The developmental history teaches that the smaller nonnucleated plates originate from cubical epithelial-cells that become flattened by inspiration, that is, by the inflation of the alveoli and the stretching of the alveolar wall. The larger plates are formed by the subsequent blending of several smaller ones. The alveoli of old embryos and of stillborn children contain only cubical cells. The walls of the alveolar ducts and of the alveoli, in addition to the previously-mentioned muscle-fibers in the former, are composed of a delicate fibrous framework and many elastic fibers. The latter are circularly arranged in the alveolar ducts; at the entrance to the alveolus (the base) the elastic fibers form a thick annular band or ring, while delicate convoluted fibers occur in the entire wall of the alveolus. The elastic rings of neighboring alveoli grow together at the points of contact and thus form the alveolar septa.

The interlobular connective tissue occurring between the lobules of the lungs in the adult contains, besides fine elastic fibers and a few connective-tissue cells, black pigment-granules and minutest particles of carbon that have come there by inhalation. In children the interlobular connective tissue is more richly developed and the demarcation of the lobules more distinct.

The surface of the lung is covered by the *visceral pleura;* this is composed of connective-tissue, numerous fine elastic fibers, and on its free surface is clothed by a simple stratum of flat polygonal epithelial-

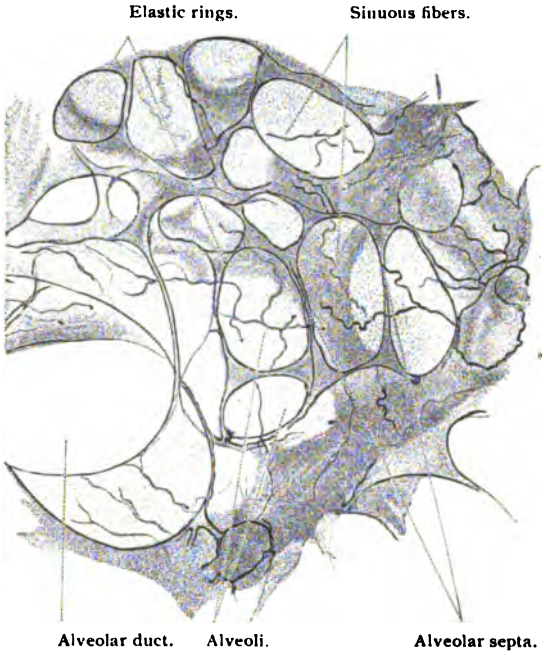



FIG. 197.—SECTION OF LUNG OF A RABBIT. × 220. Staining of elastic fibers. Techn. No. 125 *b*.

(endothelial) cells. The *parietal pleura* has the same structure, but contains fewer elastic fibers.

The *blood-vessels of the lungs*, the branches of the pulmonary artery, enter at the hilus of the lung and run beside the bronchi, bronchioles, alveolar ducts, and between the terminal vesicles, where they break up into a very narrow-meshed capillary network, that is placed immediately beneath the respiratory epithelium of the terminal bronchioles, of the alveolar ducts, and of the alveoli. The *veins* arise each at the base of an alveolus, and unite in small trunks that follow the bronchi and arteries. The walls of the bronchi are supplied by special blood-vessels, the bronchial arteries, which fur-

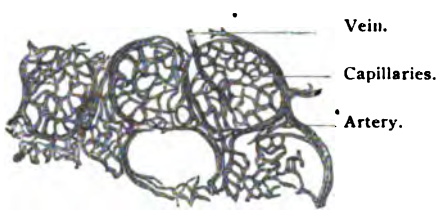



FIG. 198.—FROM A SECTION OF LUNG OF A CHILD, INJECTED THROUGH THE PULMONARY ARTERY. × 80. Of the five alveoli drawn the three upper ones are fully injected. Techn. No. 126.

nish a deep capillary plexus for the muscles and the glands, a superficial plexus for the tunica propria. These capillaries are taken up in part by the bronchial veins, in part by the pulmonary veins.

Of the *lymphatic vessels* two groups are recognized, a well-developed *superficial plexus* beneath the pleura and a wide-meshed *deep plexus* in the interlobular connective tissue. From these networks small stems furnished with valves proceed, which follow the bronchi and emerge at the hilus, where they connect with the bronchial lymph-nodules (see also p. 122).

The numerous *nerves* of the lungs, derived from the sympathetic and the vagus, contain medullated and nonmedullated nerve-fibers and small groups of ganglion-cells. The nerve-endings stand chiefly in relation to the walls of the blood-vessels.

## THE THYROID GLAND.

The thyroid body in its anlage is a compound tubular gland; its excretory canal, the thyro-glossal duct, opening at the foramen cecum of the tongue, with the exception of a few atrophic remains was oblit-

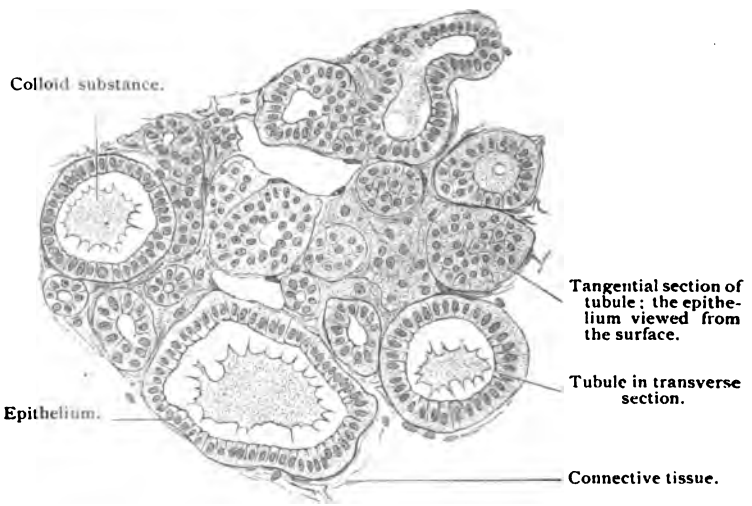



FIG. 199.—A LOBULE FROM A THIN SECTION OF THE THYROID GLAND OF ADULT MAN. × 220. The tubules vary in diameter. Techn. No. 127.

erated in an early embryonic period; the network of gland-tubules, that at first are not hollow, becomes constricted at intervals and resolves itself into short pieces, the "follicles," which become bound together into lobules by loose connective tissue. In the adult the tubules are oval sacs blind at both ends. The tubules differ greatly in diameter (from

40 $\mu$ to 120 $\mu$) and are lined by a simple layer of cubical epithelial-cells. Their contents consist of a characteristic, homogeneous, viscid mass, the *colloid substance*, which also is found in the lymph-vessels of the organ. The *blood-vessels* are exceptionally numerous and break up into capillaries that form a network around the tubules, close beneath the epithelium. The *lymphatics*, likewise profuse, form a network lying between the tubules. The *nerves* follow the ramifications of the blood-vessels and form plexuses chiefly distributed to the vascular walls, some of which also surround the gland-tubules. The penetration of terminal twigs into the epithelium has not been observed.

In the neighborhood of the thyroid body several "epithelial corpuscles," about two millimeters in size, are found; they consist of cords of epithelial-cells, capillary blood-vessels, and connective tissue, and doubtless are detached particles of the lateral anlage of the thyroid body, arrested in a certain stage of development, which in circumstances can become transformed into the genuine thyroid tissue with colloid substance.

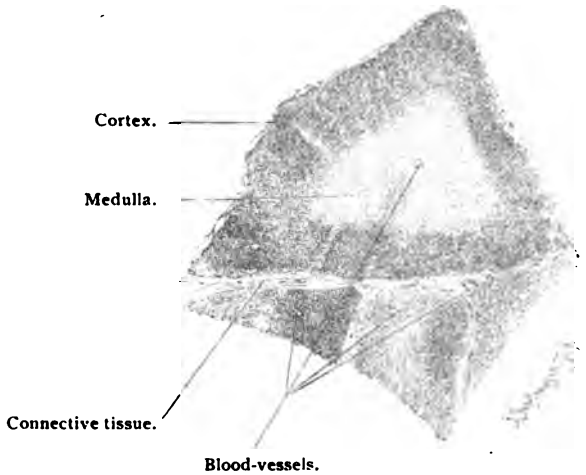



FIG. 200.—SECTION OF SECONDARY LOBULES OF THE THYMUS BODY OF A SEVEN-DAYS'-OLD RABBIT. × 50. The lower lobules are sectioned tangentially, so that chiefly only cortex is visible. Techn. No. 128.

## THE THYMUS BODY.

The thymus body, in its first anlage an epithelial organ, retains this character only during a very brief embryonal epoch, since with the exception of very small remnants the epithelium immediately undergoes degeneration, and in its place adenoid tissue appears.* The thymus in

*The adenoid tissue is not a transformation of the epithelium; it originates in the embryonal connective-tissue.

childhood consists of lobes from 4 to 11 mm. large, which are enveloped by fibrous connective-tissue mixed with fine elastic fibers. This connective tissue sends septa into each lobe, by which a subdivision into smaller (secondary) lobules 1 mm. in size is effected. Each of these lobules consists throughout of adenoid tissue, which is more densely developed at the periphery than in the center, so that a darker cortical zone can be distinguished from a lighter medullary substance. In the medullary substance concentrically-striated bodies, varying greatly in number and size (from 15 to 180 $\mu$ in diameter) are found; they are masses of altered epithelial-cells [the remains of the epithelial structures which in an embryonic stage constituted the principal bulk of the organ]. They are called *concentric corpuscles* (Hassall).

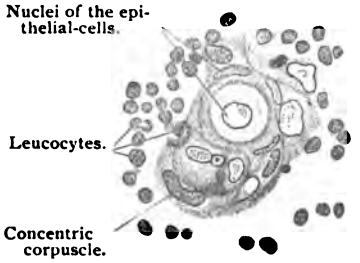



FIG. 201.—CORPUSCLE OF HASSALL FROM A SECTION OF THE THYMUS BODY OF A YOUNG DOG. × 50. Techn. No. 128.

The *blood-vessels* are very richly developed and supply a capillary system lying in the cortex and in the medulla. The *lymphatics* likewise are very numerous; the larger vessels lie on the surface of the organ, their branches run in the interlobular septa and from here penetrate into the medullary substance.

At a later period the tissue of the thymus undergoes regressive change; the greater part of the adenoid structure disappears and is replaced by fat.

## TECHNIC.

No. 122.—*The Larynx, the Bronchi and the Thyroid Gland.*—Of animals, the cat is particularly recommended. Expose the bronchi above the manubrium; cut them and the esophagus through transversely and dissect both loose upwards (see No. 97). The tongue may be removed with these parts. The thyroid gland should be allowed to remain attached to the larynx. The whole is to be placed for from two to six weeks in 200 or 400 c.c. of Müller's fluid, then washed for one hour in running water and hardened in 200 c.c. of gradually-strengthened alcohol. In about eight days cut sections, transverse and longitudinal, through the vocal cords and through pieces of the trachea; stain them for five minutes in Hansen's hematoxylin and mount in damar. Particularly instructive are sections taken transversely through the vocal cords, in which the mucous membrane, glands, muscles, blood-vessels, nerves, and cartilage furnish material for the most varied study.

No. 123.—*The Bronchi.*—From an animal just killed (rabbit) remove the lungs, fix them in Müller's fluid and harden them in gradu-

ally-strengthened alcohol, like No. 122. In eight days cut out of the lung 1 cm. cubes that contain a portion of a longitudinally-disposed bronchus. With the scissors remove the greater part of the attached lung tissue; embed the bronchus in liver, and make thin transverse sections, which may be stained in Hansen's hematoxylin and mounted in damar (Fig. 194). The lungs of cats are less suitable than those of the rabbit, owing to the often considerable masses of fat surrounding the bronchi. This method is also applicable for the exhibition of the alveoli and the alveolar passages.

No. 124.—*The Respiratory Epithelium.*—For the demonstration of this tissue only animals just killed can be used. Young kittens (*not* newborn) are suitable; they should be killed by decapitation. The trachea and lungs should be carefully taken out and filled by means of a glass pipet with a previously-prepared solution of silver nitrate (50 c.c. of a 1 per cent. solution to 200 c.c. of distilled water). The trachea should then be tied fast and the whole placed for from one to twelve hours in the remainder of the silver solution and stood in the dark. On removing them from the silver solution, the lungs should be quickly washed with distilled water and transferred to 150 c.c. of gradually-strengthened alcohol, in which they may remain (in the dark) for an indefinite length of time. The reduction can be undertaken in an hour after the silver injection or later. For this purpose the lungs in the alcohol should be exposed to *sunlight*, in which in a few minutes they become a deep brown. With a *very sharp* razor cut thin sections, taking care not to compress the tissue. Despite the hardening in alcohol the lung tissue is still soft and allows only thick sections to be cut. Sections may be most easily cut in a direction parallel to the surface. Place the sections for from ten to sixty minutes in 5 or 10 c.c. of distilled water to which a crystal of common salt about the size of a lentil has been added, and mount them unstained in damar. It is not advisable to employ nuclear staining, since not only the nuclei of the epithelial-cells, but also those of the capillaries and other tissues are colored, and consequently the picture becomes very complicated. Orientation in such cases is not altogether easy. The investigation should be begun with the low power. The small alveoli are easily recognized; the somewhat larger spaces are alveolar ducts. The demarcation of the epithelium is on the whole finer with medium magnification (80 diameters), and by no means equally good in all places. The cubical epithelial-cells are usually colored a somewhat deeper brown. Find a good place, study it with the high power (240 diameters), and by changing the focus (elevating and depressing the tube) note the relief of the preparation; with high magnification, either only the interior or the margin of an alveolus can be distinctly seen. Fig. 196 was drawn with change of focus. The pores of the alveoli can be shown only by careful injection of lungs that have been made empty of air.

No. 125.—*Elastic Fibers of the Lungs.*—(*a*) *Fresh.*—With the scissors placed on a freshly-cut surface of the lung (the lung need not

be fresh), cut a flat piece about 1 cm. square, spread it out with needles on a dry slide, apply a cover-glass and treat with two drops of potash lye diluted one-half with water; the diluted lye destroys all parts excepting only the elastic fibers, the thickness and arrangement of which may be easily investigated with the high power (240 diameters).

(*b*) *Permanent Preparation.*—Fix 1 or 2 cm. cubes of lung in absolute alcohol (§ 4, p. 30) for forty-eight hours, stain thick sections in orcein (p. 40) and mount in damar (Fig. 197).

No. 126.—*Blood-vessels of the Lungs.*—Inject the lung from the pulmonary artery with Berlin blue; fix it in Müller's fluid, and harden it in alcohol. Cut thick sections, principally parallel to the surface of the lung (Fig. 198).

No. 127.—*The Thyroid Gland.*—Thin sections of the gland, hardened in toto (see No. 122), are to be stained with picrocarmine and mounted in damar (Fig. 199). The retracted colloid masses stain an intense yellow. Examine thick sections in glycerol, in which the lymph-vessels filled with colloid substance are often distinctly visible.

No. 128.—*The Thymus Body.*—Place the thymus body of a young animal in Müller's fluid for from two to five weeks and harden it in gradually-strengthened alcohol. Stain sections with Hansen's hematoxylin; mount them in damar (Fig. 200). Care should be taken not to confuse the cross-sections of the blood-vessels, the lumina of which change in elevating and depressing the tube (when they are not true cross-sections), with the concentrically-striated corpuscles of Hassall. The preparation represented in Fig. 201 is from a thymus body fixed in Flemming's mixture and stained with safranin.

# VIII. THE URINARY ORGANS.

## THE KIDNEYS.

The kidneys are compound tubular glands, which exclusively consist of minute tubes, the *uriniferous tubules.* The macroscopically perceptible differences between the peripheral and central portions of the organ, the so-called *cortical* and *medullary* regions, are principally determined by the course of the uriniferous tubules, the divisions within the cortex pursuing a tortuous, those within the medulla a straight course.

Each *uriniferous tubule* begins in the cortex as a spherical dilatation, *renal corpuscle* (Malpighi), which is marked off by a constriction, the *neck*, from the greatly-convoluted succeeding division, the *proximal convoluted tubule*. This passes into a straight portion, that is at first centrally directed, but soon turns back and forms a loop, *Henle's loop*, in which a *descending* and an *ascending limb* may be distinguished. The latter passes into a convoluted portion, the *intercalated* or *distal convoluted tubule* (*spiral tubule*), that as it proceeds takes a straight course and is then called *collecting tubule* (Fig. 202). The collecting tubules, during their centrally-directed course, take up other distal convoluted tubules, unite under acute angles with neighboring collecting tubules, and converge toward the apex of a renal papilla, where, diminished in number but greatly increased in diameter, they terminate in the *papillary duct.* Henle's loop-tubules and the collecting tubules are named *straight tubules* (tubuli recti). Each uriniferous tubule pursues a completely isolated course until it is taken up by a collecting tubule. The loops of Henle and the peripheral portions of the collecting tubules are grouped together as they pass toward the medulla and form the structures in the cortex known as *medullary rays* or *pyramids of Ferrein.*

The minute structure of the uriniferous tubules differs so greatly in the several divisions that a separate consideration of each division is necessary. The *renal corpuscles* (Malpighi), from 0.13 to 0.22 mm. in size, consist of a spherical plexus of blood-vessels, the *glomerulus*, and the expanded and invaginated blind initial extremity of a uriniferous tubule, the *capsule of the glomerulus* (Bowman). The glomerulus lies within the invaginated portion of the capsule, and is almost completely envel-

oped by it. Accordingly, two layers are distinguished in the capsule, an inner (quasi visceral) lying close upon the glomerulus, and an outer

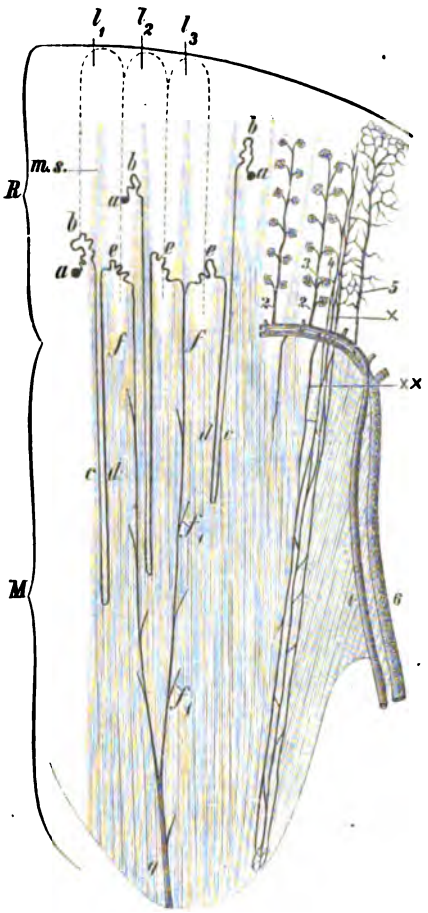



FIG. 202.—SCHEME OF THE COURSE OF THE URINIFEROUS TUBULES (LEFT) AND OF THE RENAL BLOOD-VESSELS (RIGHT), sketched from a section of kidney of an infant seven weeks old. × 10. *R*. Cortex. *M*. Medulla. *m. s*, Medullary rays. $l_1$, $l_2$, $l_3$, Three renal lobules. *a*, Renal corpuscle; *b*, proximal convoluted tubule; *c*, descending, *d*, ascending limb of Henle's loop-tube; *e*, distal convoluted tubule (spiral tubule); *f*, collecting tubule; $f_1$, portions of collecting tubules; *g*, excretory duct. 1. Branch of renal artery. 2. Interlobular artery. 3. Afferent artery. 4. Efferent artery. 5. Interlobular vein. 6. Branch of renal vein. x and xx, Branches supplying the medulla.

FIG. 203.—ISOLATED URINIFEROUS TUBULES OF A FOUR-WEEKS'-OLD RABBIT. × 30. *a*, Malpighian corpuscle. *b*, Proximal convoluted tubule. *c*, Henle's loop, descending limb; *d*, ascending limb. *f*, Collecting tubule. *g*, Papillary duct. Techn. No. 129.

(quasi parietal) layer; the former, in young animals, is composed of cubical cells, that later become more and more flattened, the latter of flat polygonal cells (Fig. 205).

At the neck of the capsule the outer layer passes over into the walls of the *proximal convoluted tubule*, which is from 40 to 60 $\mu$ thick.

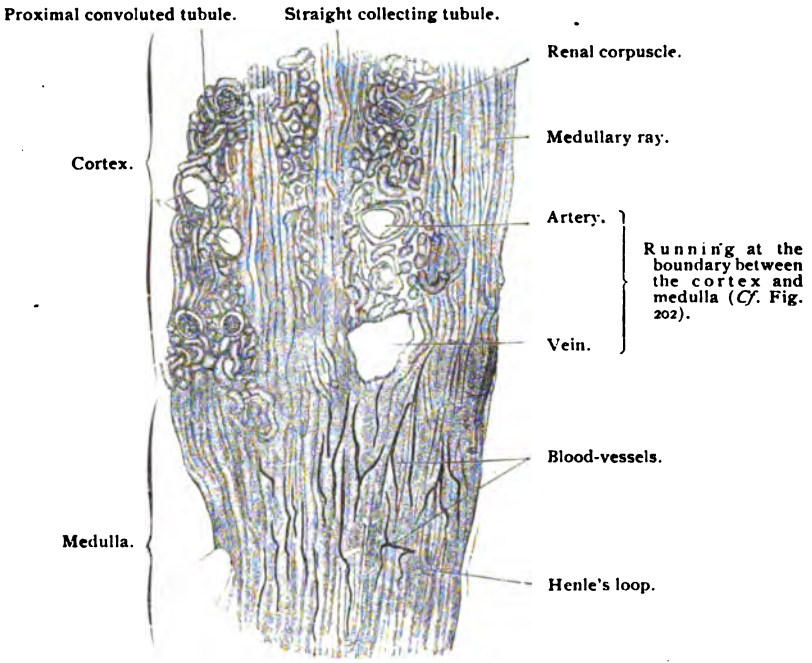



FIG. 204.—FROM A SECTION OF HUMAN KIDNEY, INCLUDING A PORTION OF THE CORTEX AND THE MEDULLA. At x two renal corpuscles have fallen out. × 20. Techn. No. 130.

The protoplasm of the cells of this division, the boundaries of which are not sharply defined, consists of granules that by means of proto-

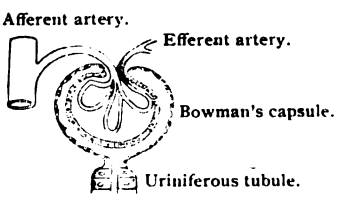



FIG. 205.—SCHEME. On the left is an artery that on the right gives off an afferent vessel; this breaks up into branches which turn into the radicles of the efferent vessel (directed toward the right). The three loops are intended to represent the glomerulus; this lies in Bowman's capsule, of which both layers are visible; below, the capsule passes into the uriniferous tubule.

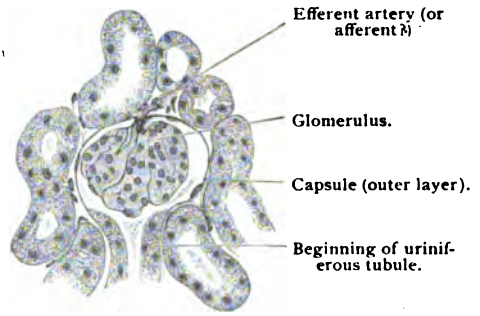



FIG. 206.—FROM A SECTION THROUGH THE CORTEX OF THE KIDNEY OF A MOUSE, SHOWING THE CONNECTION BETWEEN BOWMAN'S CAPSULE AND THE URINIFEROUS TUBULE. × 240.

plasmic filaments are bound together in rows radially placed to the lumen; these rows are most distinctly seen at the base of the cell and

with medium magnification have the appearance of minute rods (Fig. 207). The nuclei of the cells always lie near the base; the surface of the cell that is directed toward the lumen is in some places provided with an extremely unstable striated border (p. 66), the "brush-border."

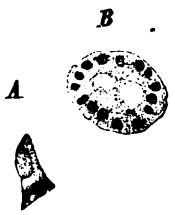



FIG. 207.—*A*. ISOLATED CELL OF A PROXIMAL CONVOLUTED TUBULE. The base of the cell is separated into minute rods. *B*. Transverse section of a proximal convoluted tubule; the rods appear as delicate striæ. Both preparations are from a cat's kidney. × 240. Techn. No. 130.

The *descending limb* of Henle's loop is from 9 to 15 μ thick; the lumen is very wide. It is lined by squamous epithelial-cells, the nuclei of which often protrude into the lumen (Fig. 209). The *ascending limb* is from 23 to 28 μ thick, the lumen relatively narrower. The epithelial-cells resemble those of the convoluted divisions, but are somewhat lower (Fig. 209). The transition of the narrow descending limb into the thicker ascending portion does not always occur at the loop. The *intercalated* or *distal convoluted portion* (spiral tubule) is from 39 to 46 μ thick; the epithelial-cells are cylindrical or conical in shape and have a peculiar luster. The *collecting tubules* increase in thickness as they approach the apex of the papilla; the thinnest have a diameter of 45 μ, the thickest (the papillary duct) a diameter of from 200 to 300 μ. Their epithelial-cells are in part clear, in part granular columnar elements, that increase in height with the increase of the diameter of the tubule (Fig. 209).

The uriniferous tubules are covered in their entire length by a structureless membrana propria situated outside of the epithelium, which is thickest in the descending limb of Henle's loop. The tubules are enveloped by a small amount of loose connective tissue (interstitial connective tissue), which on the surface of the kidney is condensed and forms a fibrous investment (tunica albuginea) containing smooth muscle-fibers. The blood-vessels run in the interstitial connective tissue.

The *blood-vessels of the kidneys*. The renal artery divides in the hilus of the kidney into branches, the interlobular arteries (*arteriæ interlobares*), which after giving off small twigs to the capsule and to the calices of the kidney enter the parenchyma of the organ at the circumference of the papillæ and without branching pass to the boundary between the cortex and the medulla (Fig. 202). Here the arteries bend at right angles and form arches (*arteriæ arciformes*) along the boundary line, with the convexity toward the periphery. From the convex side of the arches, and from their terminal ramifications, at regular intervals spring branches

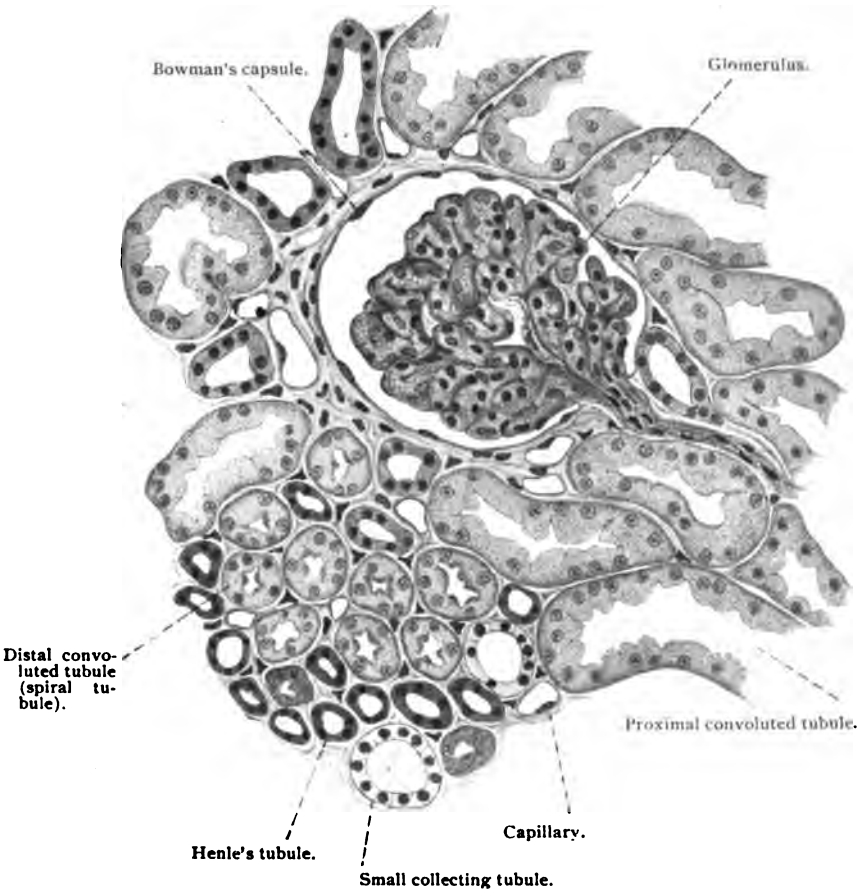



FIG. 208.—FROM A SECTION THROUGH THE CORTEX OF A HUMAN KIDNEY (parallel to the surface). At the left lower corner there is a cross-sectioned medullary ray. × 200. (Schaper.) Techn. No. 130.

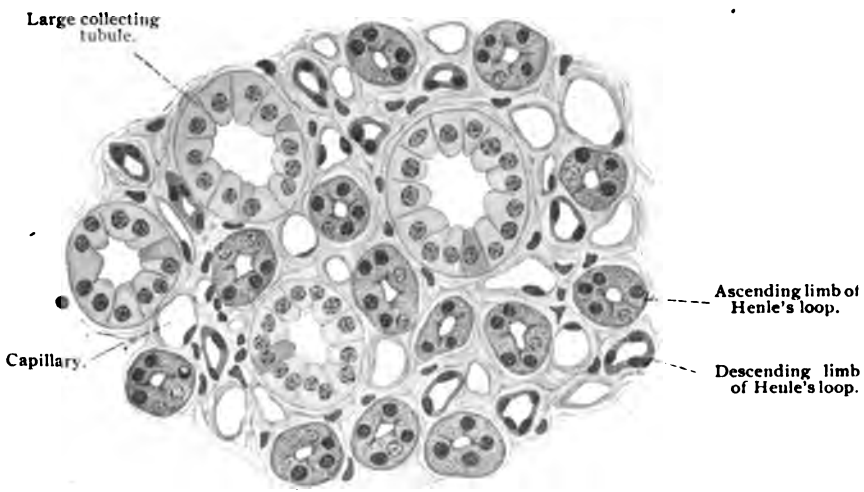



FIG. 209.—FROM A TRANSVERSE SECTION THROUGH THE MEDULLA OF A HUMAN KIDNEY. × 320. (Schaper.) Techn. No. 130.

running toward the periphery, the *interlobular arteries* * (arteriæ interlobulares), which give off short lateral twigs, each of which supplies a glomerulus (Fig. 202, 2, and Fig. 210). Each interlobular artery breaks up into terminal branches, that in part supply the capsule, in part continue as the capillaries of the cortex or form the *afferent vessel* (Fig. 202, 3) of a glomerulus. Each glomerulus arises by the rapid division of the *afferent artery* into a number of small twigs, that immediately reunite in a single arterial vessel,† called the *efferent artery*, which is somewhat smaller than the entering vessel (Fig. 202, 4, and Fig. 210). The efferent

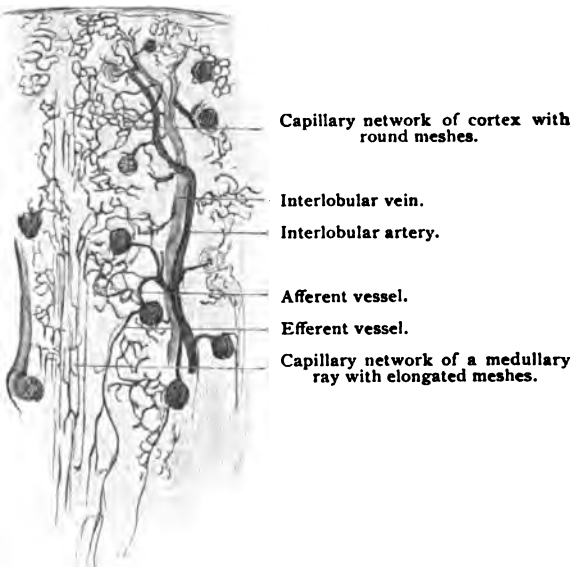



FIG. 210.—FROM A LONGITUDINAL SECTION OF THE INJECTED KIDNEY OF A GUINEA-PIG. × 30. Techn. No. 133.

artery breaks up into a capillary network with round meshes in the region of the convoluted tubules, with elongated meshes in the region of the medullary rays. From the latter veins arise, the *interlobular veins* (venæ interlobulares), which lie close beside the interlobular arteries, retrace the course of the arteries, and open into the venæ arciformes; the latter also take up small veins that arise from the confluence of capillaries situated

* Microscopic regions of the kidney with ill-defined boundaries, in the axis of which lies a medullary ray and at the periphery of which interlobular arteries ascend, are designated lobules. In Fig. 202 three lobules, $l_1$, $l_2$, $l_3$, are indicated by dotted lines. These lobules have no relation whatever to the lobules of the kidney during fetal life.

† Consequently each glomerulus is an arterial rete mirabile (see p. 122). In dogs and cats retia mirabilia occur in the kidneys that do not stand in any relation to uriniferous tubules, that is, they are not enveloped in a capsule.

in the deeper portions of the cortex. The vessels of the peripheral zone of the cortex converge to points where they unite in radicles arranged in a stellate form, the *venæ stellatæ*, which join the interlobular veins (Fig. 202, 5, and Fig. 210). The foregoing account of the distribution of the blood-vessels applies only to the cortex and to the medullary rays.

The medulla receives its blood supply from (1) the *arteriolæ rectæ*, which arise from the arterial arches at the juncture of the medulla and the cortex, from the efferent vessels of the most deeply-situated glomeruli, and direct from centrally-running branches of the interlobular arteries or of the arciform arteries ; and (2) from offshoots of the cortical capillaries (Fig. 202, x, xx). The veins of the medulla take their origin from the wide-meshed

FIG. 211.—SECTION OF THE KIDNEY OF A MOUSE. × 180. Techn. No. 134.

capillary network surrounding the papillary ducts and join the venous arches at the juncture of the medulla and the cortex. The renal vein and its branches have no valves. Direct communications between the arteries and veins occur both in the capsule and in the interior of the kidney.

The *lymph-vessels* run in part superficially, in the capsule, and in part accompany the arteries in the parenchyma of the organ. The *nerves* form plexuses which envelop the arteries as far as the renal corpuscles (Fig. 211). The convoluted uriniferous tubules are said to be surrounded by nerve-fibers, extremely delicate branches of which pierce the membrana propria and terminate in free endings between the epithelial-cells.

## THE URETERS.

The *ureters*, the *calices*, and the *pelvis* of the kidney are composed of three coats, (1) the mucous coat, which lies innermost, (2) the muscular coat, and (3) surrounding this the outer fibrous coat (Fig. 212).

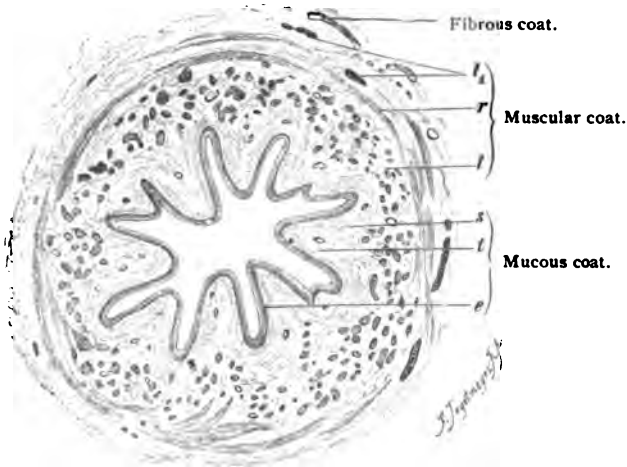



FIG. 212.—TRANSVERSE SECTION OF THE LOWER HALF OF A HUMAN URETER. × 15. *e*, Epithelium; *l*, tunica propria; *s*, submucosa; *l*, inner longitudinal muscle-bundles; *r*, circular layer of muscle-bundles; $l_1$, accessory outer longitudinal muscle-bundles. Techn. No. 135.

The tunica propria of the *mucous membrane* consists of delicate connective-tissue fibers, which, richly interspersed with cellular elements,

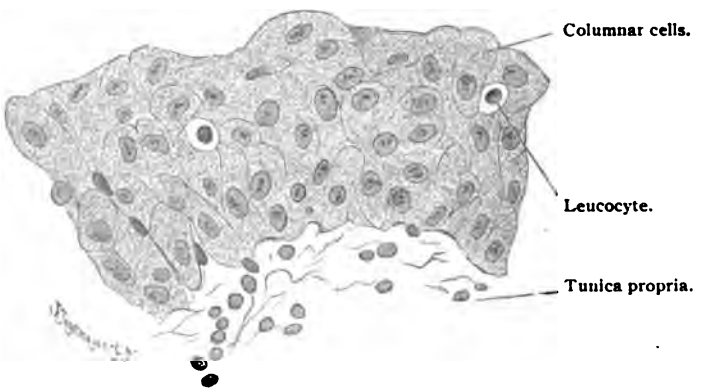



FIG. 213.—PORTION OF A VERTICAL SECTION OF A HUMAN VESICAL MUCOUS MEMBRANE. × 560. Techn. No. 136.

pass without sharp demarcation into the tissue of the submucosa. The epithelium covering the tunica propria is the so-called "*transitional*

*epithelium"*; that is, a stratified scaly epithelium composed of but few layers, of which the uppermost layer consists of cylindrical or cubical, only slightly-flattened elements. Occasionally, instead of the latter, large plate-like cells are present, which contain several nuclei that have arisen by amitotic division (p. 60, remark †).

The *muscular coat* consists of an inner longitudinal and an outer circular layer of smooth muscle-fibers, which in the lower half of the ureter possesses an additional discontinuous outer layer of longitudinally-arranged muscle-bundles.

The *fibrous coat* consists of loosely-united connective-tissue bundles.

The mucous membrane of the calices is continued over the surface of the renal papillæ, the circular muscle-fibers form a sphincter muscle around the papillæ.

The *blood-* and the *lymph-vessels* are especially numerous in the mucous coat. The *nerves* are principally distributed to the muscular coat; single fibers extend into the tunica propria as far as the epithelium.

## THE URINARY BLADDER.

The urinary bladder likewise consists of a mucous, a muscular, and a fibrous coat. The epithelium resembles that of the ureter and the pelvis of the kidney in every particular; a distinction from these is *impossible*. The tunica propria occasionally contains solitary lymph-nodules. The muscular coat consists of strata of smooth muscle-fibers, an inner and an outer longitudinal layer, which enclose between them a circular layer. The layers interlace in such a manner that it is not possible to define their exact limits. At the base of the bladder the inner longitudinal layer is augmented, the circular layer forms the not always distinct internal vesical sphincter. Blood- and lymph-vessels comport themselves as in the ureter; microscopic groups of ganglion-cells are situated along the course of the nerves.

In the tunica propria of the lower division of the pelvis of the kidney, of the upper portion of the ureter, and of the bladder round or oval bodies occur, that have been erroneously regarded as glands. They are sprouts of the surface epithelium, possess the same structure, are without a lumen, and occasionally even have severed their connection with the superficial epithelium.

## THE URETHRA.

The *female urethra* is composed of a mucous coat and a robust muscular coat. The tunica propria consists of delicate fibrous connective

tissue containing numerous connective-tissue cells and is elevated in numerous papillæ, that are especially well developed near the meatus. The epithelium varies, in some individuals it is a stratified scaly, in others a simple columnar epithelium. A few branched simple tubular glands are present; they occur in small groups at the meatus and are called "periurethral" glands. The muscular coat consists of an inner longitudinal and an outer circular layer of nonstriped muscle-fibers, between which extends a compact fibrous connective tissue containing many elastic fibers. The mucous coat is richly supplied with veins, the networks of which extend into the longitudinal layer of the muscular coat; in this way a structure similar to the corpus cavernosum of the male urethra, the *corpus spongiosum*, is formed.

The *male urethra* (better, male urogenital sinus) is likewise composed of a mucous coat and a muscular coat, but they vary in structure in the different parts of the canal. In the prostatic portion the epithelium resembles that of the bladder; in the membranous division it gradually passes into the stratified columnar variety, which in the spongy part is transformed to a simple columnar epithelium. From the fossa navicularis on, the epithelium is of the stratified squamous type. The tunica propria is rich in elastic fibers and is beset with papillæ, that are especially well developed in the fossa navicularis. Isolated branched simple tubular glands, the urethral glands (*glandulæ urethrales*, Litrii), occur throughout the entire urethra. The muscular coat in the prostatic division consists of an inner longitudinal and an outer circular layer of smooth muscle-fibers; both layers are still well defined in the membranous portion, but gradually cease in the spongy portion, where the circular layer, still conspicuous in the bulbus urethræ, is the first to disappear; in the anterior part of the spongy division a few oblique and longitudinal bundles occur (Fig. 221). The mucous membrane has a rich vascular supply (see corpus cavernosum urethræ, p. 297). The lymph-vessels lie beneath the blood-vessels.

## TECHNIC.

No. 129.—*Isolated Uriniferous Tubules.*—The most suitable for this purpose are the kidneys of young animals, for example newborn kittens. Divide the kidney in halves; place one half (*a*) aside for investigation fresh; cut the other half (*b*) into pieces including the cortex and medulla, and place them in 30 c.c. of pure hydrochloric acid.

*a.* Tease a pea-sized piece in a drop of 0.75 per cent. salt solution. The red glomeruli, the convoluted and straight uriniferous tubules, can be seen with the low power. The convoluted tubules are dark and granular, the other divisions clear. With high magnification, the nuclei

of the clear portion of the uriniferous tubules can be distinctly seen; the cell boundaries may best be seen in the collecting tubules. In the convoluted tubules only the fine striation of the bases of the gland-cells can be seen; cell boundaries and nuclei are not visible.

*b.* In about two hours the red pieces of kidney tissue should be transferred to a capsule containing 50 c.c. of distilled water, in which they rapidly turn a dirty gray and acquire smeary surfaces. The water is to be changed. After a few moments small pieces can be detached with needles and readily separated into tubules, in a little water on a slide. If it is desired to obtain entire uriniferous tubules, transfer pieces of kidney 2 cm. square to a watch-glass in which has been placed a cover-glass and enough distilled water to cover the surface of the latter. The tubules should now be isolated with needles. If the isolation is successful—this may be ascertained by examination with the low power—with filter-paper carefully absorb the water from the watch-glass and then from the cover-glass, take out the latter, cleanse its free surface, and place it with the attached tubules gently on a slide on which a drop of dilute glycerol has been previously placed. The preparation may be subsequently stained under the cover-glass with picrocarmine (Fig. 203).

No. 130.—*The Cortex and Medulla.*—For sections, the kitten's other kidney or other pieces of kidney tissue 2 or 3 cm. square are to be fixed in 200 or 300 c.c. of Müller's fluid for four weeks, or in Zenker's fluid for forty-eight hours, and hardened in 100 c.c. of gradually-strengthened alcohol. Fixation in absolute alcohol (like No. 132) is still better. Thick transverse and longitudinal sections through the cortex and similar ones through the medulla are to be examined unstained in dilute glycerol, with a low power. Thin transverse sections through the apex of the papillæ for the excretory duct, through the base of the papillæ (Fig. 209), and through the cortex are to be stained with Hansen's hematoxylin and mounted in damar. Endeavor to cut radial sections through the cortex and the medulla, showing the boundary between the two; examine them unstained in glycerol, with the low power. Frequently the blood-vessels are still filled with blood-corpuscles and may be traced for long distances. The extremely delicate "brush-borders" can be seen only here and there, with very high magnification. Frequently they have fallen off.

No. 131.—*Medullary rays and Henle's loops* are especially fine in stained vertical sections of the kidneys of young animals prepared after No. 130.

No. 132.—For the study of the *glomeruli and Bowman's capsule*, also the connection of the latter with the uriniferous tubule, the kidney of the mouse is most suitable. Fix and harden the divided kidney in 15 c.c. of absolute alcohol, which should be changed in an hour. After three days (or later) cut thin sections of the cortex, stain them two or three minutes in Hansen's hematoxylin and mount in damar (Fig. 206). The invaginated portion of the capsule, on account of the similarly-stained nuclei of the blood-vessel walls, cannot be distinguished.

No. 133.—*The Blood-vessels of the Kidney.*—An isolated kidney may be injected (p. 43) and fixed in 300 c.c. of Müller's fluid for four weeks and then hardened in 150 c.c. of gradually-strengthened alcohol. The venæ stellatæ can be investigated macroscopically. Unstained thick longitudinal and transverse sections should be studied with the low power (Fig. 210).

No. 134.—*Nerves of the Kidney.*—Treat small pieces according to Golgi's method given on p. 41; they should remain from three to six days in the osmiobichromate mixture.

No. 135.—*The Pelvis of the Kidney and the Ureters.*—Of the former pieces 1 cm. square, of the latter 1 or 2 cm. long should be fixed in Müller's fluid and in fourteen days hardened in 100 c.c. of gradually-strengthened alcohol. Stain sections with Hansen's hematoxylin and mount in damar.

No. 136.—Treat the *Bladder* like No. 135.

No. 137.—*Epithelial Cells of the Pelvis of the Kidney, of the Ureter, and of the Bladder.*—Place pieces of these parts, 1 cm. square (cut open the ureter), in 30 c.c. of Ranvier's alcohol (p. 20). Isolate and stain with picrocarmine (p. 38). Mount in diluted acidulated glycerol (p. 48).

No. 138.—*The Female Urethra.*—Cut out a piece of the female urethra about 2 cm. long, together with the attached anterior vaginal wall; place it in 100 or 200 c.c. of Müller's fluid for fixation, and in two or three weeks harden it in gradually-strengthened alcohol (p. 33). Stain cross-sections in Hansen's hematoxylin (p. 36) and mount in damar (p. 45).

No. 139.—*The Male Urethra.*—Treat pieces 1 or 3 cm. long of the prostatic, membranous, and cavernous portions and of the fossa navicularis like No. 138. Care should be exercised not to confuse the urethral lacunæ (Morgagni), blind evaginations of the mucosa, with sections of glands.

# IX. THE REPRODUCTIVE ORGANS.

## THE MALE REPRODUCTIVE ORGANS.

### THE TESTICLE.

The testicles are glands consisting of branched, pouch-like tubules, the *seminiferous tubules*, which are enveloped in a connective-tissue capsule. This capsule, the *tunica albuginea*, is a tough membrane, which encloses the parenchyma on all sides and on the posterior upper aspect is thickened, forming a mass, the *mediastinum testis* (*corpus Highmori*), which juts into the interior of the organ. From this a number of septa arise, which pass along divergent paths to the tunica albuginea and so divide the parenchyma of the testicle into *pyramidal lobules*, the base of which is directed toward the capsule, the apex toward the corpus Highmori. The tunica albuginea consists of dense fibrous connective tissue, which on its free surface is covered by a simple layer of flat epithelial-cells,* on its inner surface is in contact with a layer of loose connective tissue; this supports numerous blood-vessels and is called *tunica vasculosa;* it is connected with the interlobular septa. The mediastinum, a dense connective-tissue structure, contains a network of freely-anastomosing tubules, the *rete testis* (Haller). The interlobular septa consist of bundles of connective tissue, continuous with the connective tissue surrounding the individual seminiferous tubules. This "interstitial" connective tissue is rich in cellular elements, which are in part flat connective-tissue cells, in part spherical cells, the so-called *interstitial cells*, containing pigment or fatty granules, in man also crystalloids.

The *seminiferous tubules* in their course may be divided into three portions: they begin as (1) the *convoluted tubules*, which pass into (2) the *straight tubules*, which continue as (3) the *rete testis*. The convoluted tubules are round, winding canals, about 140 $\mu$ in diameter, of which the initial extremity has not yet been definitely located; probably they are united with one another at the periphery, beneath the tunica vasculosa, and so form a network from which numerous tubules turn aside and with

* This is the visceral layer of the tunica vaginalis propria.

many windings pass toward the mediastinum. Tubules with blind ends have been observed. During their course the tubules diminish in number,

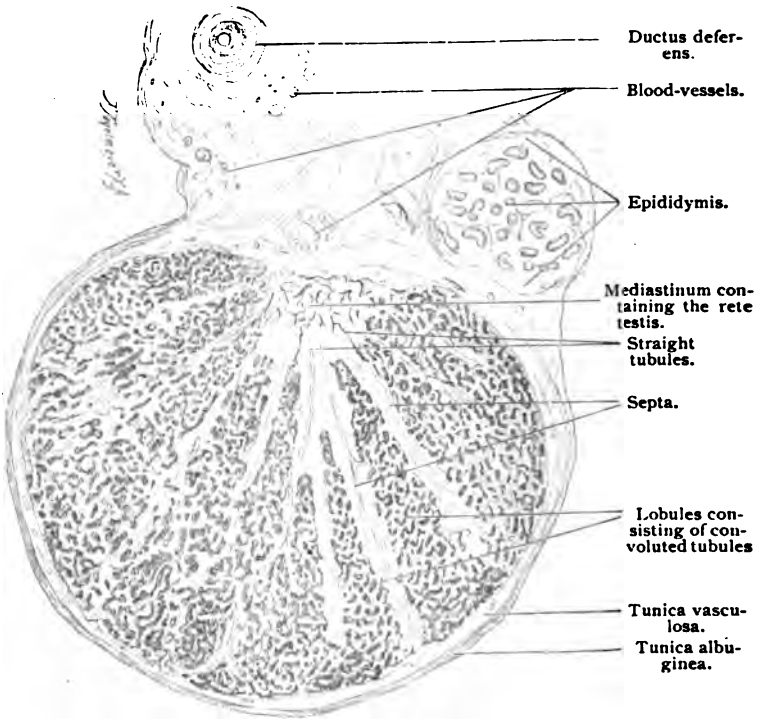



FIG. 214.—CROSS-SECTION OF THE TESTICLE OF A NEWBORN CHILD. × 10. Techn. No. 140.

FIG. 215.—FROM A CROSS-SECTION OF THE TESTICLE OF AN OX. × 50. In the processes of fixing and hardening the epithelium has become somewhat shrunken, so that spaces occur between it and the interstitial connective tissue. Techn. No. 141.

because they continually unite with one another under narrow angles. Not far from the mediastinum the convoluted tubules pass into the straight tubules, which considerably reduced in size (20 to 25 $\mu$ thick), after a

short course penetrate into the mediastinum and form the rete testis, the tubules of which measure from 24 to 180 $\mu$ (Fig. 214).

The walls of the *convoluted tubules* from without inward consist of (1) several layers of flattened endothelioid connective-tissue cells, (2) a thin membrana propria, and (3) of a stratified epithelium, the character of which varies greatly in the several divisions of the tubules. When the gland is in a state of rest several strata of spherical cells, the nuclei of which stain more or less intensely, may be seen lining the tubules (Fig. 215).

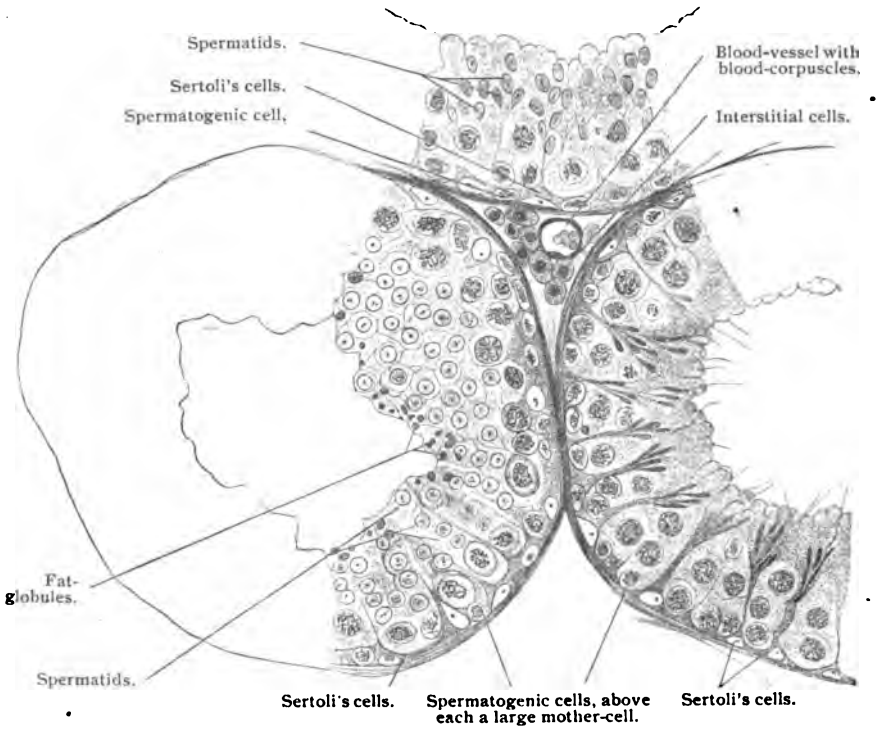



FIG. 216.—CROSS-SECTION OF SEMINIFEROUS TUBULES OF A MOUSE. × 360. Observe that the nuclei of the spermatids (below on the left) at first round, become oval (above) and are transformed (below on the right) into the heads of the seminal filaments. Techn. No. 142.

In a state of activity the epithelium exhibits a cycle of phenomena relating to *spermatogenesis*. The cells lying next to the basement membrane, the *parietal stratum*, are of two kinds, the *sustentacular cells* or *Sertoli's columns*, which take no direct part in the production of the seminal filaments, and the *spermatogenic cells* (ancestral cells), the real producers of the semen (Fig. 216). They multiply by indirect division and grow to be large cells, that occupy the next layer within. These are the *mother-cells*, which divide twice, each giving rise to four *daughter-cells* lying in

a zone still nearer to the center of the tubule. The latter are the *spermatids* and from them the spermatozoa are directly derived. The nucleus of each spermatid develops into the head of a spermatozoon, a small portion of the protoplasm forms the caudal filament. The middle-piece reacts like paranuclein and probably is derived from the centrosome. While these changes are in progress the columns of Sertoli grow in length centrad and a large number of spermatids form a connection with each one of them;* it is highly probable that by means of this "copulation" the spermatids receive their nutritive material.

The walls of the *tubuli recti* consist of a membrana propria and within this of a simple layer of low columnar cells.

The canals of the *rete testis* are lined by a simple stratum of cubical or flat epithelial-cells.

The *arteries* of the testicles are branches of the spermatic artery, which proceed in part from the mediastinum and in part from the tunica vasculosa to the intertubular septa, and there break up into capillary networks which surround the seminiferous tubules. The *veins* arising from these networks follow the course of the arteries. The *lymph-vessels* form a plexus beneath the tunica albuginea, which is in connection with the network of lymph-capillaries enveloping the seminiferous tubules. The *nerves* form networks about the blood-vessels; whether single fibers branch off from these networks, pierce the membrana propria, and terminate in club-shaped endings between the epithelial-cells is not yet definitely established.

## THE SEMEN.

The secretion of the testicles, the semen, almost exclusively consists of *spermatozoa*, pin-shaped structures in which a *head* and a *tail* are distinguished (Fig. 217). In man the *head* is from 3 to 5 $\mu$ long and from 2 to 3 $\mu$ broad, flattened, viewed from the side pyriform in shape, with the tapering end directed forward, seen from surface oval, with the anterior end rounded and containing a clear portion (Fig. 217, 1). The *tail* when very highly magnified exhibits a filament extending from end to end, the *axial fiber*, which is composed of delicate fibrils. Three divisions are recognized in the tail: the round *middle-piece*, lying next to the head, 6 $\mu$ long and scarce 1 $\mu$ broad; following this the *main-piece*, from 40 to 60 $\mu$ long, gradually diminishing in thickness posteriorly; the tip

* Whence the "spermatoblast" of authors, see Techn. No. 143, p. 317.

of the tail, the *end-piece*, is about 10 $\mu$ long and consists of the projecting axial fiber.*

The spermatozoa are distinguished by their extraordinary vitality (probably due to the calcareous substances which they contain).

The sinuous movements of the spermatozoa are executed by the cilium alone, which propels the head before it; they seldom occur in the concentrated secretion of the testicle and first begin only after dilution normally effected by admixture of the fluids of the ampullæ, of the seminal vesicles, of the prostate gland, and of the bulbo-urethral glands. In this mixture of fluids the motions may continue for from twenty-four to forty-eight hours after death and for a still longer period in the secretions of the female genitalia. Water paralyzes the movement, which, however, may be restored by the addition of normal animal fluids of alkaline reaction and moderate concentration; normal fluids in general, also a one per cent. salt solution, exert a favorable influence on the vibrations of the spermatozoa, while acids and metallic salts suspend them. In motionless spermatozoa the caudal filament is frequently looped (Fig. 217, 3).

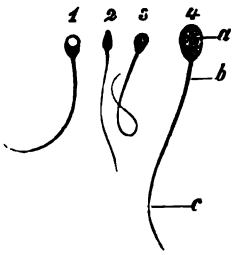



FIG. 217.—1, 2, 3. HUMAN SPERMATOZOA. × 360. 1. Viewed from the surface. 2. Viewed in profile. 3. Coiled seminal filament. 4. Spermatozoon of ox; *a*, head; *b*, middle-piece; *c*, main-piece. The end-piece and the demarcation of these parts cannot be perceived with this magnification. Techn. No. 144.

## THE EXCRETORY DUCTS OF THE TESTICLE.

The excretory ducts of the testicle include the epididymis, the ductus deferens, the seminal vesicles, and the ejaculatory duct. (The tubuli recti and rete testis belong to the excretory ducts, but were described with the gland because they are enclosed within it.) From the upper end of the rete testis about fifteen *ductuli efferentes testis* emerge, which by their progressively-increasing convolutions form as many conical lobules, the *lobuli epididymidis*. The aggregate of the lobuli constitute the so-called *head* of the epididymis. By the union of the *ductuli efferentes* the *ductus epididymidis* arises, which with its complex convolutions forms the body and tail of the epididymis and then continues as the *ductus deferens*.

* The forms of spermatozoa in different animals cannot be described here. In birds and tailed amphibians a spiral fiber, united to the axial fiber by a hyaline membrane, has been discovered; it has also been found in the rat and other mammals, but has not been demonstrated with certainty in man.

The *ductuli efferentes* are lined by an epithelium consisting of totally dissimilar varieties; groups of simple ciliated cylindrical elements alternate with clusters of cubical cells without cilia; consequently the latter have the appearance of simple saccular glands, that, however, do not produce evaginations of the membrana propria (Fig. 218). A fibrous membrana propria and a tunic of nonstriped muscle consisting of several circular strata complete the walls of the ductuli efferentes.

The *ductus epididymidis* possesses a stratified ciliated epithelium; its convolutions are supported and held together by a loose, vascular connective tissue; toward the ductus deferens the circular strata of muscle-fibers increase in thickness (Fig. 218).

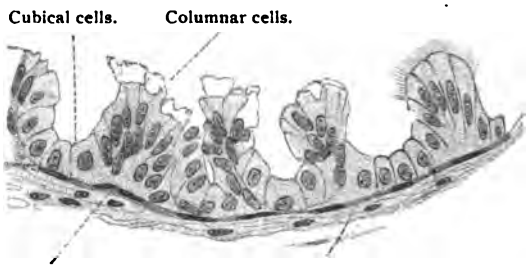



FIG. 218.—TRANSVERSE SECTION OF AN ADULT HUMAN DUCTULUS EFFERENS TESTIS. The right-hand end of the illustration is schematic. No cilia could be seen, although those of the epithelium of the epididymis were well preserved. Techn. No. 147.

The *ductus deferens* consists of either a two-layered columnar epithelium or of a transitional epithelium, a layer of connective tissue divided into a tunica propria and a submucosa, an inner circular and an outer longitudinal stratum of smooth muscle-fibers, and a fibro-elastic adventitia. The latter, notably in the division lying between the testicle and the ejaculatory duct, contains longitudinally-disposed bundles of smooth muscle-fibers * (Fig. 219). In the initial portion of the ductus deferens there also is a thin layer of longitudinal nonstriped muscle-fibers in the submucosa. The terminal portion expands forming the *ampulla*, the walls of which are thinner, but otherwise exhibit a similar structure. In the mucous membrane of the ampulla there are branched gland-follicles; the columnar cells of the epithelium contain numerous pigment-granules. The *seminal vesicles* have the same structure. The *ejaculatory duct* consists of a simple columnar epithelium and thin inner

* They really belong to the tunica vaginalis of the spermatic cord (funiculus spermaticus), and are known as the musculus cremaster internus.

circular and outer longitudinal strata of smooth muscle-fibers, as well as an adventitia containing dense venous plexuses.

Excepting the networks around the blood-vessels, the *nerves* form an intricate plexus provided with sympathetic ganglia, the *plexus myospermaticus*, situated in the muscularis of the epididymis and in that

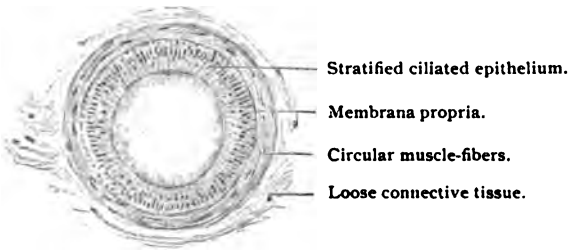



FIG. 219.—TRANSVERSE SECTION OF A HUMAN DUCTUS EPIDIDYMIDIS. × 80. Techn. No. 147.

of the ductus deferens, where it is even more dense, from which delicate fibers continue into the mucous membrane.

The *paradidymis* (Giraldès), lying between the convolutions of the epididymis and the *ductulus aberrans* (Haller) are atrophic remains of the embryonal mesonephros. Both consist of tubules lined by ciliated cubical or cylindrical epithelium and enveloped by a vascular

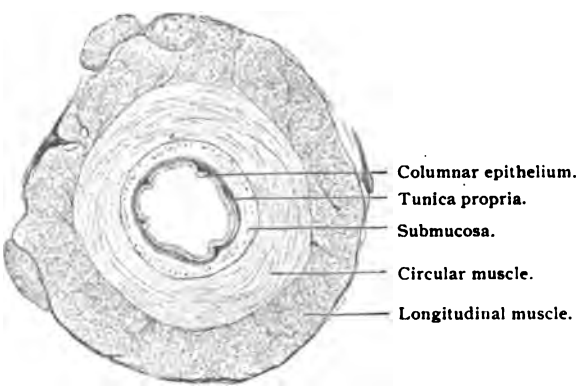



FIG. 220.—TRANSVERSE SECTION OF THE INITIAL PORTION OF A HUMAN DUCTUS DEFERENS. × 240. The transversely-cut longitudinal muscle-fibers of the submucosa appear as minute circles and dots. Techn. No. 147.

connective tissue. The *appendix testis* or *hydatid of Morgagni* is a solid lobule composed of a highly-vascular connective tissue and covered by a ciliated columnar epithelium; it possesses a short pedicle, which contains a duct lined by ciliated columnar epithelium. The inconstant *appendix epididymidis* is a vesicle lined by cubical epithelial-cells and

contains a clear fluid. The meaning of these appendices has not yet been fully explained; it is uncertain whether they are remains of the anterior end of the embryonal Müllerian duct, that in the female becomes the fallopian tube, or remnants of the primitive kidney.

## THE PROSTATE BODY.

The prostate body consists for the lesser part of glandular tissue, for the greater part of nonstriped muscle-fibers. The glandular portion is composed of from thirty to fifty simple branched tubular serous glands, which are characterized by their loose structure, that is, by the wide intervals between the tubules. The tubules open by two large and a number of smaller ducts into the urethra. The glandular cells are low columnar elements, which in a simple layer line the tubules. In the larger ducts the epithelium is of the transitional variety, like that in the prostatic portion of the urethra. In elderly persons the so-called *prostatic crystals*—round stratified masses of secretion up to 0.7 mm. in size—occur in the gland-tubules. The involuntary muscle-fibers, found in large quantities everywhere between the gland-lobules, are augmented toward the urethra and form a robust circular layer (the internal vesical sphincter muscle); numerous involuntary muscle-fibers are also found on the external surface of the prostate body, where they are contiguous to the bundles of striated muscle-fibers of the musculus sphincter urethræ membranaceæ.* The prostate gland and the colliculus seminalis are provided with many blood-vessels. Regarding the terminations of the numerous nerves nothing is definitely known.

The *glandulæ bulbo-urethrales* (Cowper) are compound tubular glands, the wide tubules of which are clothed with a simple layer of clear columnar cells, the excretory duct of which is lined with two or three strata of cubical cells.

## THE PENIS.

The penis consists of three cylindrical bodies: the two corpora cavernosa and the corpus spongiosum, which are enveloped by fascia and skin.

Each corpus cavernosum is composed of a fibrous sheath, the tunica albuginea, and of erectile tissue. The *tunica albuginea* is a stout connective-tissue membrane, possessing an average thickness of 1 mm.,

* Both sphincters are now designated musculus prostaticus.

intermingled with many elastic fibers, in which an outer longitudinal and an inner circular layer may be distinguished.

The *erectile tissue* is established by lamellæ and trabeculæ of connective tissue containing bundles of smooth muscle-fibers, that by means of numerous anastomoses form a network the spaces of which are lined by a single stratum of flat epithelial-cells. The spaces are filled with venous blood. The thick-walled arteries in part pass into capillaries, in part open directly into the deep cortical plexus. The capillaries form a network beneath the tunica albuginea, the *superficial* (*fine*) *cortical plexus*, which is connected with a many-layered net of wider venous channels, the *deep* (*coarse*) *cortical plexus*. The latter lies

FIG. 221.—FROM A TRANSVERSE SECTION OF THE CAVERNOUS PORTION OF THE HUMAN URETHRA. × 20. *l*, Urethral glands; the lowermost line indicates the fundus of the gland, the upper lines, portions of the excretory duct; *g*, blood-vessels; *m*, transverse section of longitudinally-disposed muscle-fibers; *r*, superficial cortical capillary network. Techn. No. 148.

in the superficial strata of the erectile tissue and gradually passes into the venous spaces of the same. The so-called *helicine arteries* are small branches lying within slender connective-tissue cords, which protrude as loops in the cavernous spaces and in an imperfect injection appear to terminate in blind ends. The *veins* which return the blood from the corpora cavernosa partly arise from the deep cortical plexus, partly from the deeper portions of the erectile tissue. They penetrate the tunica albuginea and empty into the dorsal vein of the penis.

The *corpus spongiosum* consists of two different divisions; the central portion is formed by a reticulum of the conspicuously-developed veins of the submucosa of the urethra; the peripheral portion resembles

in structure the corpora cavernosa, excepting that there is no direct communication of the arteries with the venous spaces. The tunica albuginea is composed of a layer of circularly-arranged bundles of fibrous tissue. The glans consists of greatly-convoluted veins, that are held together by a well-developed connective tissue, the carrier of the arterioles, and by the capillaries.

## THE FEMALE REPRODUCTIVE ORGANS.

### THE OVARIES.

The ovaries consist of connective tissue and glandular substance. The compact connective tissue, the *ovarian stroma*, is arranged in several strata; outermost lies the *tunica albuginea*, a structure composed of two or more intersecting lamellæ of connective tissue, which pass by imperceptible gradations into the stroma of the *cortex;* the latter encloses the glandular substance and is continuous with the *medulla*,

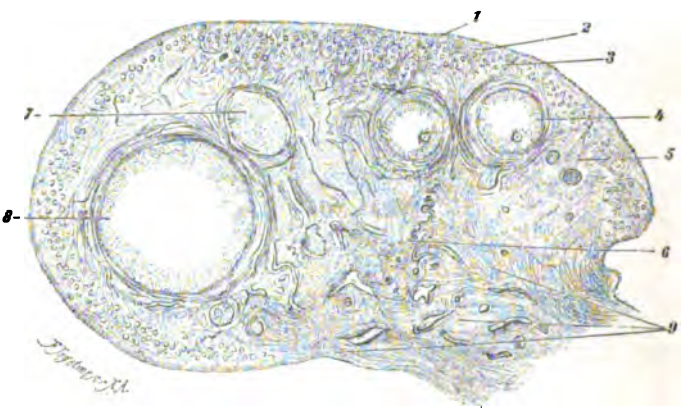



FIG. 222.—TRANSVERSE SECTION OF THE OVARY OF A CHILD EIGHT YEARS OLD. × 10. 1. Germinal epithelium; 2, tunica albuginea, as yet but slightly developed; 3, outermost zone of the cortex containing numerous minute follicles; 4, larger follicle; 5, inner division of cortex; 6, medulla with numerous tortuous arteries; 7, follicle cut at the periphery; 8, large follicle, the cumulus ovigerus not within the plane of the section; 9, hilus, containing wide veins. Techn. No. 149.

which contains numerous convoluted blood-vessels and strands of smooth muscle-fibers accompanying them. The *glandular substance* is formed by a profusion of spherical epithelial sacs, the *egg-follicles*, each of which contains an ovum. In the human ovary there are about 36,000 follicles. The majority of the follicles are microscopic in size (40 $\mu$) and in the outermost stratum of the cortex form an arched zone embracing the entire organ except at the hilus, where the vessels

and nerves enter. The larger follicles occupy the deeper portions of the cortex. The largest, those follicles readily perceptible by the unaided eye, when fully developed extend from the medulla to the tunica albuginea. The surface of the ovary is covered by a simple layer of very small, mostly short cylindrical cells, the *germinal epithelium.*

Only the initial stage in the development of the ova takes place during the embryonal period; their subsequent development, from the primordial to the fully-ripened follicle, may be observed in all its phases in every functionally active ovary. During the fetal period many cells of the germinal epithelium divide into two cells lying one above the other, of which the lower enlarges and becomes the *primordial ovum* with its conspicuous nucleus and nucleolus, while the upper cell and also its neighbor-cells become flattened and apply themselves scale-like around the ovum. Such conditions are still found after birth (Fig. 223). The ovum, which under circumstances may divide once more, surrounded by its indifferent neighbor-cells, moves down into the ovarian stroma, while above in the germinal epithelium new primordial ova arise in the same way, that likewise move into the depths. Thus originate entire complexes of egg-cells and indifferent cells of the germinal epithelium, which are named *egg-balls* (egg-pouches, egg-nests). Each ovum subsequently becomes separated from its neighbor by the rapid multiplication of the indifferent epithelial-cells, as well as by proliferation of the connective tissue, and is then an isolated, spherical body, the *primitive follicle*, that consists of the ovum and the epithelial-cells enclosing it, the so-called follicular epithelium, and of a connective-tissue sheath. So far the processes are chiefly fetal. The cells of the follicular epithelium now grow taller (Fig. 224), then

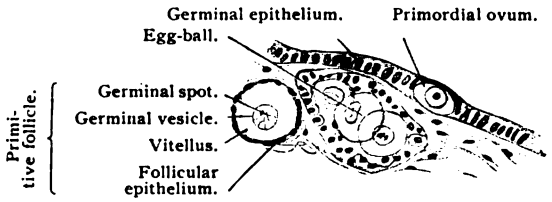



FIG. 223.—FROM A VERTICAL SECTION OF AN OVARY OF AN INFANT FOUR WEEKS OLD. × 240. The primordial ovum has a large nucleus with a nucleolus. The egg-ball contains three ova, surrounded by cylindrical cells. Techn. No. 149.

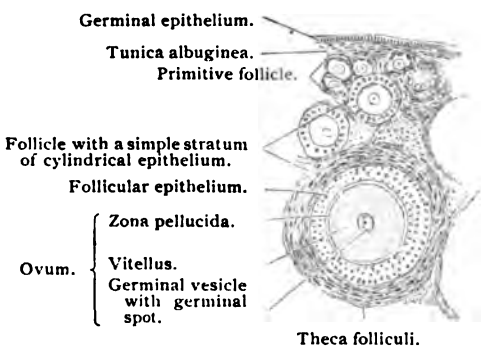



FIG. 224.—FROM A SECTION OF THE CORTEX OF THE OVARY OF A RABBIT. × 90. Techn. No. 149.

become stratified, the ovum grows larger, takes up an eccentric position within the follicle, and acquires a delicate, radially-striated border-membrane that gradually increases in thickness, the *zona pellucida* (oölemma). With the enlargement of the ovum a differentiation of its protoplasm is also completed; the greater portion of it is transformed into a crummy mass, the *deutoplasm;* of the original protoplasm, the *egg-protoplasm*, there remains only a zone around the eccentrically-situated nucleus and a thin stratum covering the surface of the ovum. The deutoplasm and egg-protoplasm are together named *vitellus;* the nucleus is called *germinal vesicle* (vesicula germinativa), which contains the *germinal spot* * (macula germinativa). Ameboid movements have been observed in the latter.

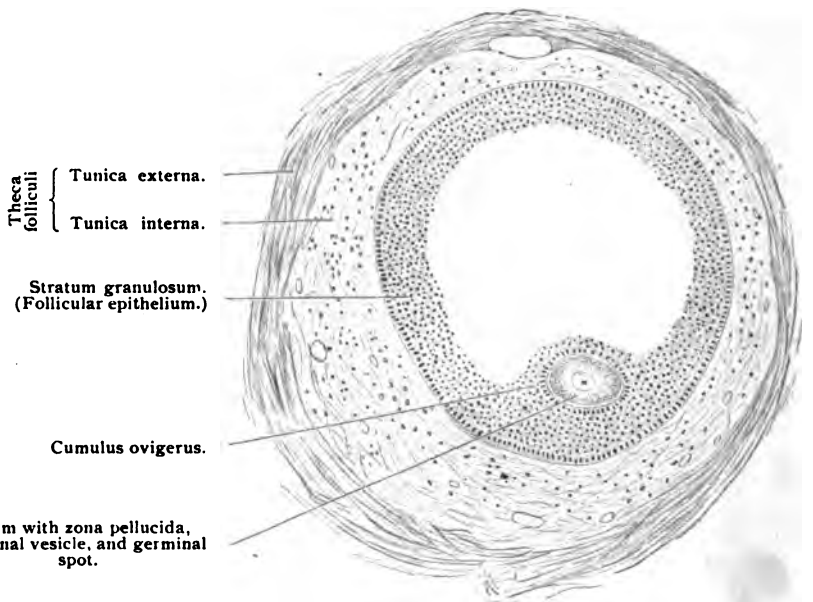



FIG. 225.—SECTION OF A LARGE GRAAFIAN FOLLICLE OF A CHILD EIGHT YEARS OLD. × 90. The clear space within the follicle contains the liquor folliculi. Techn. No. 149.

The follicle now develops further; during continual multiplication of the cells of the follicular epithelium a cleft appears in their midst that becomes filled with a fluid substance, the *liquor folliculi.* This liquid is partly a transudate from the blood-vessels surrounding the follicle, partly derived from the liquefaction of some of the cells of the follicular epithelium; it progressively increases in quantity and the follicle expands to a vesicle, the *Graafian follicle* (folliculus vesiculosus), having a diameter of from

* The germinal spot cannot be regarded as a nucleolus, since it differs from this in its chemical relations. It is not composed of paranuclein, but of a substance resembling nuclein.

0.5 to 12 mm. Around the larger follicles the connective tissue of the stroma is arranged in circular strands forming a sheath called the *theca folliculi* (Fig. 224), in which an outer fibrous layer, the *tunica externa*, and an inner vascular layer rich in cells, the *tunica interna*, may be distinguished (Fig. 225). The stratified follicular epithelium, which in teasing fresh follicles becomes detached in large shreds, has long been known as the *stratum* (*membrana*) *granulosum;* at one point it presents a thickening, the *discus proligerus* or *cumulus ovigerus*, which encloses the ovum. The cells of the cumulus which lie next to the zona pellucida are radially placed to the ovum and form the *corona radiata* (Fig. 226). The

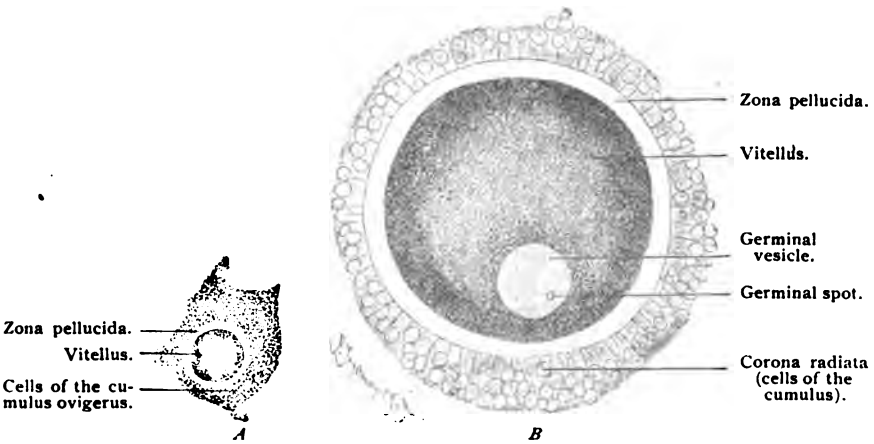



FIG. 226.—AN OVUM FROM THE GRAAFIAN FOLLICLE OF A COW. *A* magnified 50, *B* magnified 240 times. The radial striation of the zona pellucida cannot be seen. Techn. No. 150.

greater part of the interior space of the follicle is occupied by the liquor folliculi.

When the vesicular follicle has attained its full development, it bursts at the pole directed toward the surface of the ovary, where its site is previously indicated by the attenuated and arched overlying tissue; the ovum enveloped by the cumulus escapes into the pelvic cavity, the empty follicle undergoes regressive change and is converted into the yellow body, the *corpus luteum.* When the discharged ovum is not fertilized the yellow body disappears after the lapse of a few weeks; it is then called the *false corpus luteum;* if on the other hand the escape of the ovum is followed by pregnancy, the ruptured follicle develops into the *true yellow body*, which possesses a diameter of about one centimeter and endures for years. It consists of a fibrous membrane (the former tunica externa) and of a yellow mass that has arisen by the enlargement of the fatty, metamorphosed cells of the follicular epithelium and by

proliferation of the cells of the tunica interna; the latter are transformed into delicate connective-tissue septa, that penetrate between the cells of the follicular epithelium (Fig. 227). In the center of the corpus luteum there occasionally is a cavity filled with blood. The blood is derived from the torn vessels of the tunica interna. Later the center becomes decolorized and the blood-clot is replaced by a crummy mass occasionally containing hematoidin crystals (see p. 121).

Not all the primitive follicles attain complete development. Many undergo regressive change. Retrograde metamorphosis of mature follicles also occurs. This process is effected as follows: first the ovum dies and then cells, in part elements of the stratum granulosum, in part

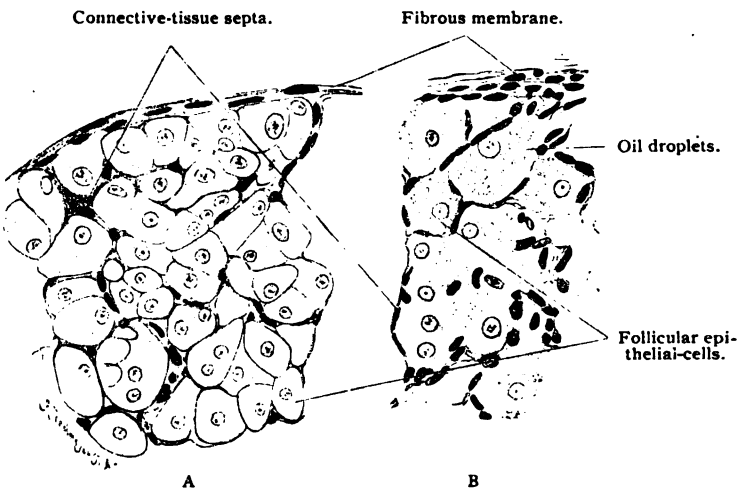



FIG. 227.—A. Portion of a corpus luteum of a rabbit. B. Portion of a corpus luteum of a cat. × 260. In B the follicular epithelium is undergoing fatty degeneration and the cells are filled with oil-droplets.

leucocytes, wander into the ovum and liquefy and absorb its substance. Having completed the disintegration and resorption of the vitellus, the migrated cells perish.

The *arteries* of the ovary, branches of the ovarian and the uterine arteries, enter at the hilus, divide in the medulla, and are characterized by their tortuous course (Fig. 222). From the medulla they pass to the cortex, where they chiefly supply capillary networks situated in the tunica interna of the follicles. The *veins* form a dense plexus at the hilus of the ovary. The *lymph-vessels* are numerous and may be traced to the tunica interna of the follicles. Medullated and nonmedullated *nerves* in large number enter at the hilus in company with the blood-vessels, to the walls of which the majority of them are distributed. A

few of the nerves proceed to the cortex; these form there a dense plexus of delicate, mostly gray fibers, which embraces the follicle and sends minute fibrils to the walls of the blood-vessels; whether nerve-fibers penetrate within the epithelium of the larger follicles is not yet definitely established.

The *epoöphoron* and the *paroöphoron* are remains of embryonal structures. The former lies within the lateral portion of the broad ligament (mesosalpinx) at the hilus ovarii (in the cat, mouse, etc., within the hilus) and consists of a group of convoluted blind tubules, the walls of which consist of ciliated columnar epithelium and circularly-arranged connective-tissue fibers. The epoöphoron is the remains of the middle or sexual segment of the primitive kidney. The paroöphoron lies in the median division of the mesosalpinx and consists of branched tubules lined with ciliated columnar epithelium; it represents the posterior segment of the mesonephros.

## THE OVIDUCT.

The walls of the *oviduct* or *fallopian tube* consist of three coats: an inner *mucous*, a middle *muscular*, and an outer *serous*. The *mucous membrane* is thrown into numerous longitudinal folds, so that on transverse section the lumen of the narrow portions of the oviduct has a stellate outline. The folds correspond in amplitude to the size of the tube and are highest in the ampulla, where they are united to one another by minute oblique secondary plications. The thick *mucous coat* is composed of (1) a fibro-elastic tunica propria containing numerous connective-tissue cells, (2) of a layer of simple ciliated columnar epithelium, the ciliary wave is directed toward the uterus, (3) of an extremely thin muscularis mucosæ consisting of longitudinally-disposed bundles of smooth muscle-fibers, and (4) of a submucosa formed by a thin layer of fibrillar connective-tissue. The *muscular coat* consists of an inner thicker circular and an outer very thin longitudinal layer of involuntary muscle-fibers. The *serous tunic* is formed by the peritoneum and by a considerable layer of loosely-united connective-tissue bundles.

The *blood-vessels* are especially abundant in the mucosa, where they form a narrow-meshed capillary network. The larger veins run along the base of the longitudinal folds of the mucosa. The knowledge of the exact behavior of the *lymph-vessels* is still wanting. The nerves form a rich plexus in the mucosa, from which branches ascend to the epithelium. A penetration in the epithelium has not been observed.

## THE UTERUS.*

The walls of the uterus, like those of the oviduct, consist of a mucosa, a muscularis, and a serosa.

The *serosa* exhibits no special characteristics.

The *muscularis* consists of smooth muscle-fibers, united into bundles which interlace in all directions, so that a sharp demarcation of single layers is not possible; still in general three strata, more or less well-defined, may be distinguished: (1) an *inner layer* (stratum submucosum), chiefly composed of bundles disposed in a longitudinal direction; (2) a *middle layer*, the most robust, consisting of bundles having in general a

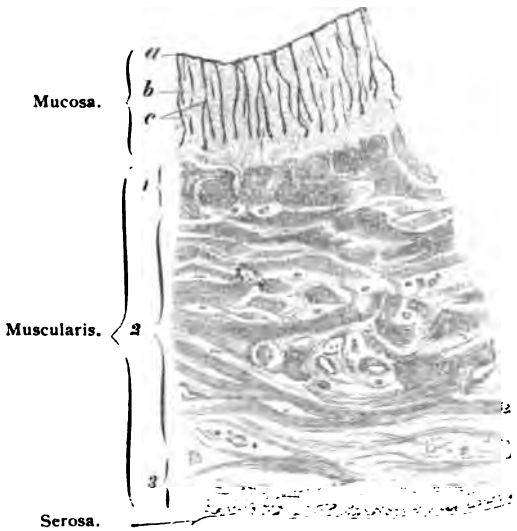



FIG. 228.—FROM A TRANSVERSE SECTION OF THE MIDDLE OF THE UTERUS OF A GIRL FIFTEEN YEARS OLD. × 10. *a*, Epithelium; *b*, tunica propria; *c*, glands; 1, inner muscular layer (stratum submucosum); 2, middle muscular layer (stratum vasculare); 3, outer muscular layer (stratum supravasculare). Techn. No. 153.

circular disposition and containing the principal ramifications of the arteries, also a well-developed venous plexus, hence the name *stratum vasculare;* (3) an *outer layer* (stratum supravasculare), formed of bundles extending partly in a circular, partly in a longitudinal direction, the latter close beneath the serosa, with which they are intimately united. The stratification of the muscular tissue is more pronounced in the cervix, where an inner and an outer longitudinal may be distinguished from a middle circular layer. The volume of the muscularis is subject to great variation, dependent on the functional state of the uterus.

* This chapter has been revised and considerably enlarged by the editor.

The *muscle-fibers* differ somewhat from the elements of smooth muscle-tissue as found in other organs. They are elongated cells, usually spindle-shaped, or are blunted and frayed at the ends. Frequently they are forked at their extremities. Their length varies greatly, in the virgin uterus from 40 to 60 $\mu$; during pregnancy they increase excessively and at the end of the same attain a size of from 300 to 600 $\mu$. The *nucleus* (not infrequently two or more are present in one cell) is usually oval and lies embedded in a granular substance.

The *mucosa* is sharply defined from the muscularis. It is the coat which in the different functional states of the uterus undergoes the pro-

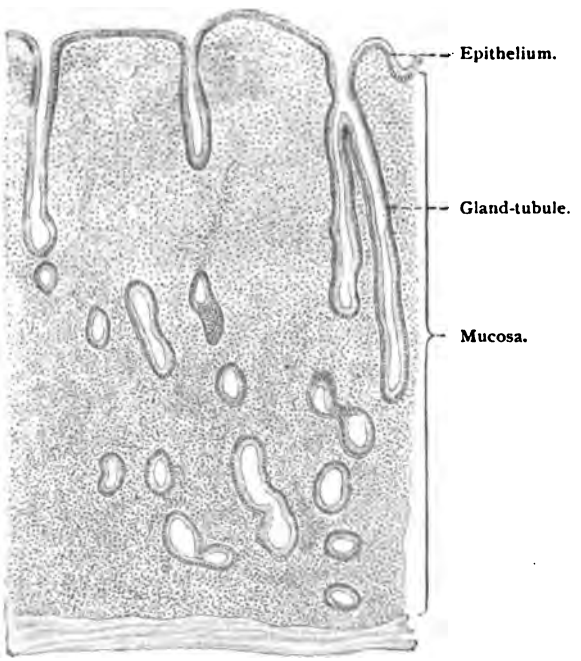



FIG. 229.—MUCOUS MEMBRANE OF THE RESTING UTERUS OF A YOUNG WOMAN. × 35. (*After Böhm and von Davidoff.*)

foundest and physiologically the most important changes. A description of the histologic structure of the mucosa of the uterus can, therefore, only answer to the corresponding functional condition of the organ, and in consideration hereof will be presented in separate sections.

It is desirable to consider :—

1. The mucosa of the virgin resting organ.
2. The mucosa of the menstruating uterus.
3. The mucosa of the gravid uterus.

The mucosa of the virgin resting uterus (Fig. 229), after the advent

of puberty, has a thickness of from 1 to 2 mm. and bears on its surface a single layer of ciliated columnar epithelium, 30 $\mu$ in height in the middle regions; the ciliary wave is directed toward the cervix. The tunica propria is formed of a fine fibrous tissue closely resembling embryonal connective tissue; it consists of elongated cells furnished with oval nuclei, which send out in all directions branched processes that unite with those of neighboring cells and so form a cellular network, the meshes of which are occupied by lymph and numerous leucocytes.

The tunica propria supports many simple or forked gland-tubules, of which the upper part pursues a course more or less vertical to the surface of the mucosa, while the lower half usually appears irregularly spiral. The glands extend close up to the muscularis and here not infrequently they are bent at right angles, so that the fundus runs parallel to the muscular coat. The glands of the uterus are to be regarded as invaginations of the superficial epithelium and likewise consist of a simple layer of ciliated epithelium resting upon a delicate basement membrane composed of anastomosing connective-tissue cells.

The blood-vessels run in a winding manner from the muscularis to the surface of the mucosa and the *arteries* in particular are characterized by their extremely-convoluted, corkscrew-like course. At the surface they break up into capillaries and form a close network. A similar network surrounds the gland-tubules. The *veins* proceeding from the capillaries form a plexus in the deeper strata of the mucosa, that is especially well developed in the cervix and particularly around the external orifice.

In the *cervix* the mucous membrane is thicker and in its upper two-thirds is clothed with a single layer of tall ciliated cells (60 $\mu$ high in the middle portion),* while toward the external orifice papillæ covered by a stratified squamous epithelium appear. In addition to a few scattered tubular glands, mucous follicles, the so-called *mucous crypts*, occur; they are 1 mm. wide, possess many evaginations, and by retention of their secretion are converted into cysts, the *ovula Nabothi*.

During the *period of menstruation* a number of progressive and regressive changes take place in the mucosa of the uterus, which may be grouped in three phases:—

(*a*) Thickening of the mucosa, accompanied by changes in its histologic structure.

(*b*) Menstruation proper.

(*c*) Regeneration.

* Transformation of these cells into goblet-cells occurs.

The *initial phase* is characterized by a considerable increase in the thickness of the mucosa (up to 6 mm.), in consequence of which the surface becomes irregular and the orifices of the glands open in deep depressions. The thickening of the mucosa depends in a measure on an actual increase of the tissue produced by proliferation of the connective-tissue cells and leucocytes and by growth of the gland-tubules,

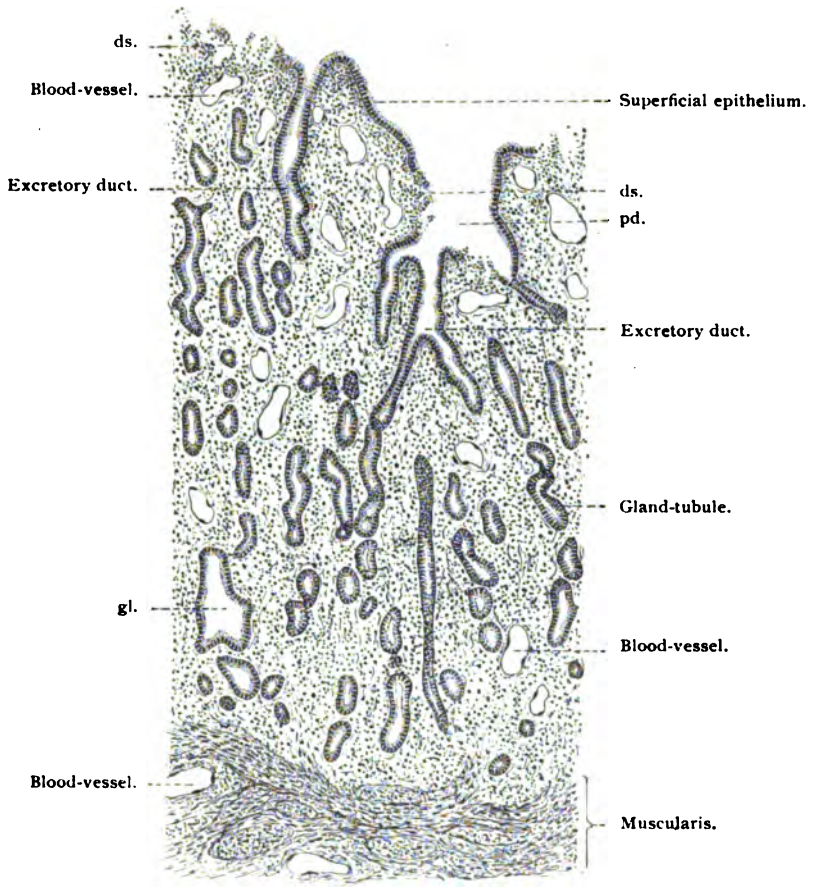



FIG. 230.—MUCOUS MEMBRANE OF A VIRGIN UTERUS DURING THE FIRST DAY OF MENSTRUATION. ds, Disintegrating surface; pd, pit-like depression of the mucous membrane; gl, glandular lumen very much enlarged. × 30.—(*Schaper.*)

which in the process take up an irregular course and become essentially wider. Simultaneously the blood-vessels, especially the veins and capillaries, undergo enormous distention, whereby the blood-supply of the organ is extraordinarily augmented. In this condition the mucosa is designated *decidua menstrualis.*

These changes are followed by a partial disintegration of the super-

ficial strata of the mucosa, accompanied by an infiltration of blood into the subepithelial tissues. The molecular disintegration (associated with fatty degeneration) of the surface advances rapidly, the greatly-dilated superficial blood-vessels become exposed, rupture, and cause hemorrhages within the uterine cavity, which flow into the vagina and give rise to the external phenomena of *menstruation*. After this discharge of blood the mucosa is rapidly reduced in thickness. The surface is now entirely devoid of epithelium and consists of connective tissue and exposed blood-vessels. This condition is immediately succeeded by the

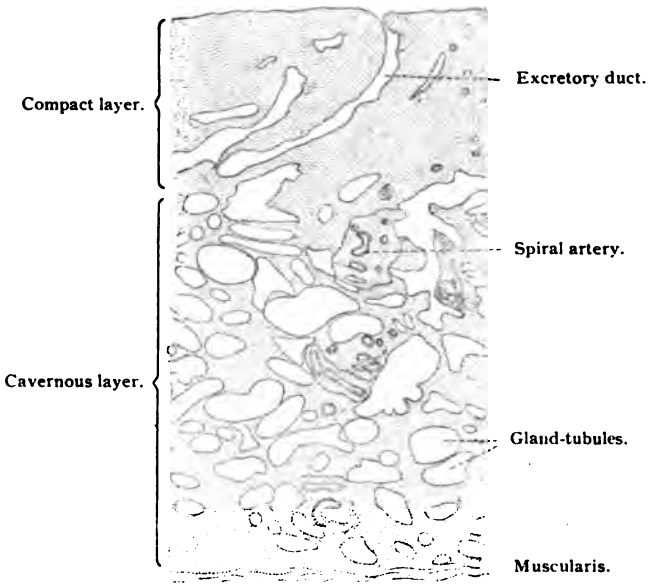



FIG. 231.—VERTICAL SECTION THROUGH THE MUCOUS MEMBRANE OF A HUMAN UTERUS ONE MONTH PREGNANT; it shows the outlines of the glands and the division of the mucosa into an upper compact and a lower cavernous layer.—(*After Minot.*)

stage of *regeneration*. The hyperemia rapidly disappears, the extravasated blood is partly resorbed, partly cast off, a cellular network grows upward and restores the lost tunica propria, while from the gland-cells the epithelial covering of the mucosa is regenerated. New subepithelial capillaries are formed.

The histology of the mucosa of the uterus during *pregnancy* (decidua graviditatis) (Fig. 231 and Fig. 232) is, on the whole, like that of the decidua menstrualis, with the alterations more pronounced. It, however, undergoes considerable modification because of its intimate relations with the developing ovum in the uterus. These relations vary,

and thus in the course of development three essentially different parts of the mucosa may be distinguished :—

(*a*) The *decidua serotina* (decidua basalis), the area of the mucosa to which the ovum is attached (placenta uterina).

(*b*) The *decidua vera*, which comprises all the remaining portion of the mucosa attached to the wall of the uterus.

(*c*) The *decidua reflexa* (decidua capsularis), the portion of the mucosa which projects into the cavity of the uterus and encapsules the ovum.

The decidua serotina and decidua vera undergo progressive develop-

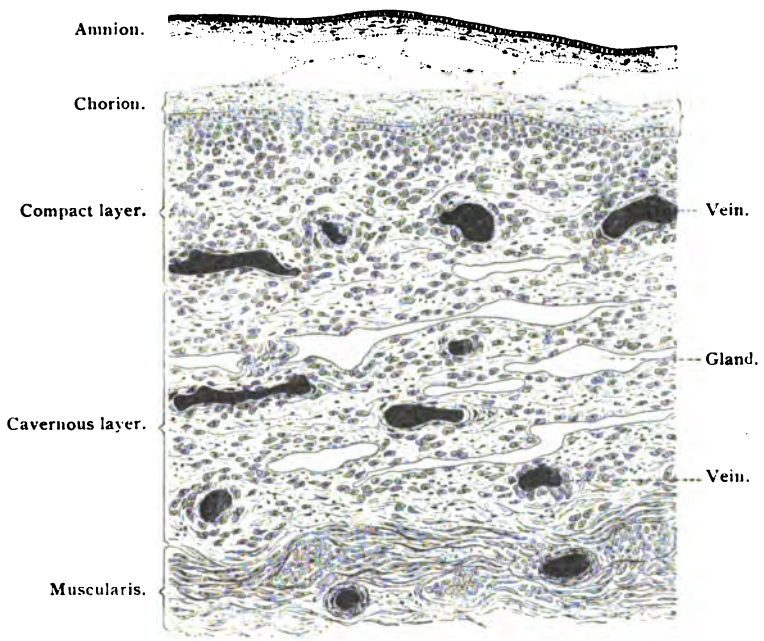



FIG. 232.—VERTICAL SECTION THROUGH THE WALL OF A UTERUS ABOUT SEVEN MONTHS PREGNANT, WITH THE FETAL MEMBRANES IN SITU. Between amnion and chorion are threads of the intermediate gelatinous connective tissue. × 30.—(*Schaper.*)

ment during the entire course of pregnancy and persist until its close ; the decidua reflexa becomes gradually attenuated and disappears in the course of the fifth month.

A section of the greatly-thickened mucosa (decidua vera and decidua serotina) shows the same histologic details that have been described in the menstrual decidua, but with this difference, that the progressive alterations (proliferation of the connective-tissue elements, distention of the blood-vessels and glands) attain much greater propor-

tions. A *superficial compact zone* and a *deep spongy zone* can always be distinguished (Fig. 231). The cavities in the latter are produced by the lower divisions of the gland-tubules, which have become greatly widened and very tortuous. At a later stage of pregnancy, owing to the great distention of the uterus, the lumina of the glands appear compressed and straighter (parallel to the muscular coat). (Fig. 232.) Between the glands are numerous blood-vessels, spindle-cells, and multinucleated giant-cells. The epithelium of the glands early begins to loosen, and in great part the cells lie irregularly scattered in the lumen of the tubule, where they disintegrate. The orifices of the glands are gradually obliterated, since the walls after the loss of the epithelium become adherent and grow together.

The *blood-vessels* of the mucosa are all dilated, especially the superficial veins and capillaries; the latter often form distended sinus-like cavities in the upper layer of the decidua. In the decidua serotina the arteries and veins open on the surface of the mucosa (Fig. 234 and Fig. 235), so that here the maternal blood circulates between the chorionic villi of the placenta (see page 316). In the decidua vera the blood-vessels, toward the end of pregnancy, are less conspicuous.

Of especial interest are peculiar, typical cells, *decidual cells*, that appear in large numbers in the mucosa of the gravid uterus. They are flattened, spherical, oval, or branched cells of conspicuous size (0.03 to 0.1 mm.), that in the latter half of pregnancy assume a characteristic brown color. They usually possess but one nucleus, though occasionally two, three, or more are present, and in rare cases as many as thirty or forty. The decidual cells are most numerous and most densely aggregated in the upper compact zone of the serotina (Fig. 232), which owes its typical character and brown color to these elements. Occasionally cells are found that are united with one another by means of protoplasmic processes. According to Minot, the decidual cells originate from connective-tissue elements, therefore may be regarded as a modified embryonal or so-called anastomosing connective tissue.

FIG. 233.— DECIDUAL CELLS FROM THE MUCOUS MEMBRANE OF A HUMAN UTERUS ABOUT SEVEN MONTHS PREGNANT. Below a "giant-cell," above to the right a cell with a karyokinetic figure. × 250.—(*Schaper.*)

In a cross-section of the decidua vera in the latter half of pregnancy, it will be seen that the surface of the mucosa is covered by two

distinct membranes—fetal membranes—the *chorion* and the *amnion* (Fig. 232). The chorion lies next to the decidua vera and is intimately united with it. It consists of two layers, an epithelial and a connective-tissue layer, of which the former is turned toward the uterine wall, the latter toward the amnion. Two similar layers may be distinguished in the amnion, but of these the epithelial layer, which consists of cubical cells, is turned toward the cavity of the uterus, while the connective-tissue stratum faces the chorion. The amnion and chorion are loosely united to each other by mucous connective tissue, in which delicate fibrils may be seen extending from one membrane to the other.

The *lymph-vessels* of the uterus form in the mucosa a wide-meshed network provided with blind branches. From this small stems proceed through the muscularis and communicate with a close subserous network of larger channels.

The *nerves* of the uterus, medullated as well as nonmedullated, are very numerous. They branch—the medullated nerves after losing their medullary sheath—in the muscularis and form a dense plexus in this and in the mucosa. From the latter delicate fibrils may be traced between the epithelial-cells.

## THE PLACENTA.*

The placenta is an organ which from a morphologic standpoint is composed of two heterogeneous parts, of which the one is produced by the mother (placenta uterina), the other by the embryo (placenta fœtalis). It is the result of the intimate union of a circumscribed area of the chorion (chorion frondosum) with that portion of the mucosa of the uterus known as the decidua serotina. The placenta serves the purpose of bringing the fetal and the maternal blood into the closest proximity, to render possible the interchange of materials between them. To subserve this function the organ possesses a peculiar histologic construction, in which the blood-vessels, especially in their arrangement and structure, take a prominent part.

In the histologic investigation of the placenta various obstacles are encountered, owing to its being an extremely soft, spongy mass, traversed by numerous wide blood-vessels. The comprehension of the minute structure will be considerably facilitated by proceeding from the previously-mentioned fact that the finished organ is the product of two originally heterogeneous structures, the chorion on the one side, the decidua serotina on the other, and that their union is substantially effected in that

* This chapter is an entirely new addition by the editor.

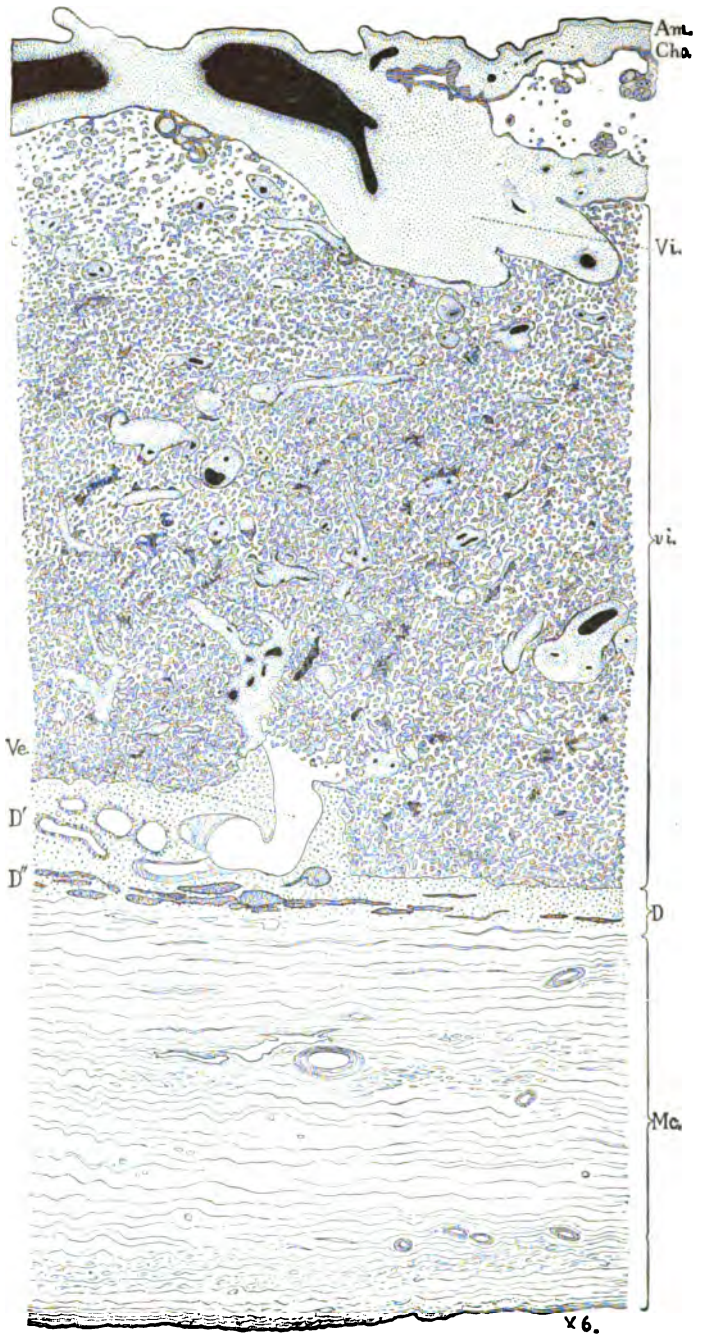



FIG. 234.—SECTION THROUGH A NORMAL HUMAN PLACENTA OF ABOUT SEVEN MONTHS, IN SITU. Am., Amnion; Cho., Chorion; Vi, trunk of a villus; vi, sections of villi in the substance of the placenta; D, decidua basalis; Mc, muscularis; D', compact layer of decidua; Ve, uterine artery opening into the placenta. The fetal blood-vessels are drawn black; the maternal blood-spaces are left white; the chorionic tissue is stippled except the canalized fibrin, which is shaded by lines; the remnants of the gland-cavities in D'' are stippled dark.—(*After Minot.*)

the chorion, by means of numerous villous-like proliferations, penetrates the underlying serotina, the surface of which is peculiarly modified and further regressively altered for its reception, and as it were takes root in the same. For the investigation of these relations sections through the wall of the uterus with the placenta *in situ*, toward the end of pregnancy, are most instructive. In such a section two sharply-defined zones may be recognized: an outer compact stratum consisting of the greatly-thickened muscular coat of the uterus, covered externally by the serosa, and an inner spongy zone containing numerous inter-communicating spaces filled with blood. The latter is the placenta, that is, the united decidua serotina and chorion frondosum. The accompanying illustration (Fig. 234) shows their relations under low magnification, which will be elucidated by referring to the schematic representation in Fig. 235.

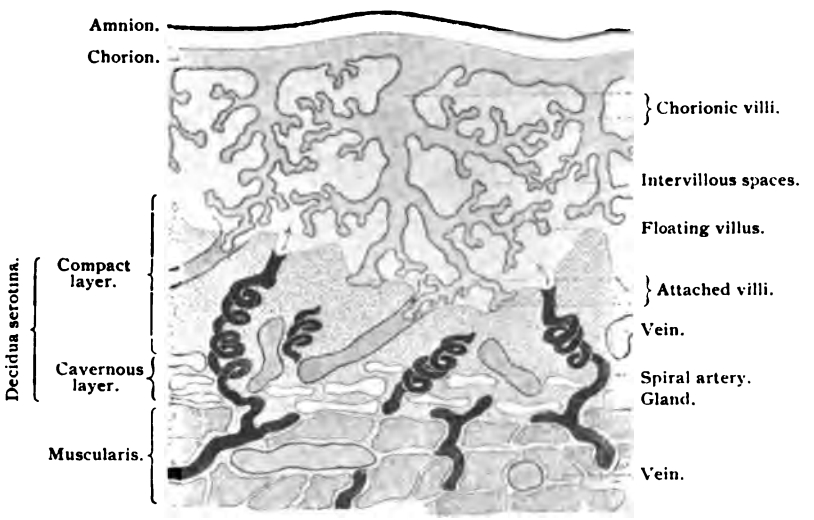



FIG. 235.—DIAGRAM OF HUMAN PLACENTA AT THE CLOSE OF PREGNANCY. Comp. Fig. 234.—(*Schaper.*)

The surface of the placenta directed toward the cavity of the uterus is covered by a compact stratum, the *membrana chorii*, which is chiefly composed of fibrillar connective tissue, in which the main branches of the umbilical blood-vessels run. The outer surface of the chorion is covered by a delicate membrane, the placental portion of the amnion, which as previously stated consists of an inner epithelial and a connective-tissue layer and is attached to the chorion by means of embryonic connective tissue. The other surface of the membrana chorii, that directed toward the wall of the uterus, is closely beset with innumerable villous-like structures, large and small, which in the upper part of the

placenta form a dense tangle, the terminal ramifications of which are embedded in the cleft, uneven substance of the serotina. On closer study of this villous tangle it will be seen that the larger stems run a more or less direct course from the chorion to the serotina, in order to secure a firm union with the latter, while their many much-branched lateral twigs usually establish no connection with the uterine portion of the placenta, but terminate free in the blood-spaces, the so-called *intervillous spaces*, between the chorion and serotina. Dependent upon these relations the branches of the chorionic villi are divided into "*roots of attachment*," or *main stems*, and *free processes*, or *floating villi*. From the chorion a branch of the umbilical artery enters each main stalk and within the terminal ramifications of the villus breaks up into a dense

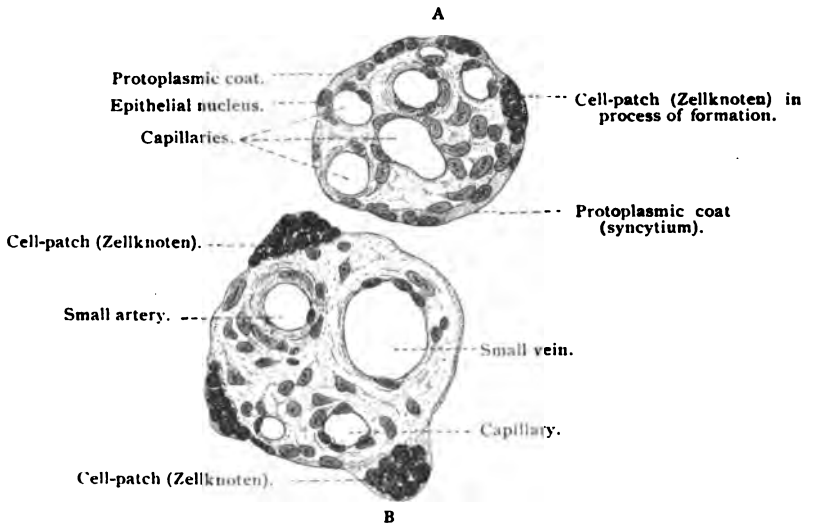



FIG. 236.—CROSS-SECTION THROUGH A SMALLER (A) AND LARGER (B) CHORIONIC VILLUS OF A HUMAN PLACENTA AT THE END OF PREGNANCY. × 250.—(*Schaper.*)

capillary network from which the umbilical veins take their origin and carry back the blood from the chorion through the umbilical cord to the fetus. *Accordingly, the blood-vessel system of the fetal placenta is entirely closed. Nowhere does a direct intermingling of the maternal and the fetal blood occur.*

A cross-section of one of the smaller chorionic villi, highly magnified, shows that it is chiefly composed of mesenchymal tissue (mucous tissue), in which the blood-vessels are embedded (Fig. 236). This central supporting substance is covered by an irregular and not everywhere continuous stratum of epithelium. In the earlier months of development two distinct strata may be distinguished in the epithelium

of the villi: an inner, lying immediately upon the supporting tissue, in which the cells possess large nuclei and definite contours, so that in the main they are distinctly separated from one another, and an outer layer, consisting of a continuous protoplasmic mass—*syncytium*—containing numerous small, irregularly-scattered nuclei. Toward the end of pregnancy, however, the epithelium of the villi undergoes great alteration, as appears in the illustration (Fig. 236). On the larger villi a true epithelial investment has almost entirely disappeared and instead isolated accumulations of large round nuclei are found; they stain intensely, are embedded in a clear, homogeneous substance, and form protuberances (*Zellknoten, cell-patches*) on the surface of the villi. Between these cell-patches the connective-tissue of the villi frequently is covered only by a thin, homogeneous stratum, or in some cases (especially in smaller villi) this stratum still retains more or less the character of the protoplasm containing scattered nuclei. There are many indications that the latter is the remains of the syncytium, while the cell-patches probably originated in the primitive inner stratum of the epithelium of the villi. In many places the syncytium is transformed into a peculiar hyaline substance permeated by fissures, which often lies upon the chorion in dense strata and is called *canalized fibrin.**

The histologic structure of the maternal portion of the placenta—*placenta uterina*—in its essential features has been described in connection with the decidua in the preceding chapter. But certain peculiarities, as well as the union of maternal and fetal placenta in a functional whole require a brief consideration.

The placental portion of the decidua (Fig. 234), that forming the lower stratum of the placenta (basal plate), is greatly thinned (from 0.5 to 1 mm.), but as in the extraplacental portion an upper compact layer and a lower cavernous layer (gland-lumina) may be distinguished. The decidual cells are extremely numerous and lie closely crowded. A honey-combed structure of connective-tissue septa (*septa placentæ*) arises from the surface of the serotina, directed toward the intervillous spaces, and penetrates between the villi of the chorion, separating the latter into lobes or cotyledons. Only in the peripheral regions of the placenta do

* It has not been yet determined with certainty whether the epithelium of the villi of the *human* placenta is entirely derived from the epithelium of the chorion, or whether the epithelium of the serotina participates in its composition. Recent investigations, however, as well as comparative anatomical facts, indicate that only the inner epithelial stratum of the villi comes from the chorionic epithelium, while the syncytium is derived directly from the mucosa of the uterus, the epithelium of which, on the ingrowth of the chorial villi, becomes closely applied to, and blends with, the epithelium of the latter.

these septa reach to the membrana chorii, where frequently they form on the inferior surface of the latter a thin membranous stratum, the *decidua placentalis subchorialis.* On the margin of the placenta the serotina gradually increases in thickness and passes into the vera, at which point it is closely applied to and firmly united with the chorion. Within the area of the placenta, however, the chorion and serotina are far apart and the space between them is filled with the above-described chorial villi and the blood circulating between them; it is *maternal* blood that surrounds the villi on all sides and is thus brought into the closest relation with the fetal circulation.

Of especial interest is the behavior of the blood-vessels within the placenta uterina (Fig. 234 and Fig. 235). Numerous *arteries* from the muscularis of the uterus penetrate the serotina, in which they make corkscrew-like tours during the course of which they lose their muscular coat and continue as wide tubes consisting alone of the lining endothelium. Near the surface of the decidua they usually bend sharply at right angles and then open directly into the intervillous spaces of the placenta.* *Nowhere do the arteries break up into capillaries.* The *veins* (likewise endothelial tubes, though wider than the arteries) also are in direct communication with the placental spaces; they enter the decidua usually under a very narrow angle, run more or less parallel to the surface, and unite in the deeper strata in a wide venous plexus. In accordance with the description of these conditions of the vessels, the arteries and veins within the serotina can no longer be recognized by the histologic structure of their walls, but can only be distinguished by their width and their course. The arteries in addition usually are characterized by a

*In regard to the relation of the decidual blood-vessels to the intervillous spaces there are two conflicting theories. According to the one the intervillous spaces are independent clefts without proper walls, that are formed in the course of development between the fetal and maternal portions of the placenta, with which the blood-vessels opening on the surface of the decidua are in direct communication. Accordingly the villi of the chorion are in direct contact with the maternal blood circulating in these spaces. The opposite view regards the blood-spaces of the placenta as the enormously-widened capillaries of the decidua, which, during the mutual process of intergrowth between the placenta uterina and placenta fœtalis, the developing villi of the chorion have invaginated. According to this the blood-vessel system of the decidua is closed and the arteries and veins communicate through a system of capillary lacunæ (the intervillous spaces). Further, the chorial villi are not directly bathed in the maternal blood, but are separated from it by a thin stratum of cells, the capillary endothelium, which lies directly upon them. Recent investigations of Keibel apparently support the latter view, since in a human placenta in an early stage of development he succeeded in tracing the endothelium of the decidual blood-vessels into the intervillous spaces and demonstrating it as a continuous stratum on the surface of the chorionic villi. It is possible that in the further development of the placenta this endothelial covering undergoes regressive change, so that in later stages it cannot as a rule be demonstrated.

thin, homogeneous, enveloping stratum that stains intensely with carmine, in which a few scattered nuclei are found. This peculiar layer is probably a product of the degenerated muscular coat.

## THE VAGINA AND THE GENITALIA.

The *vagina* is formed by a mucous membrane, a muscular tunic, and a fibrous tunic.

The *mucous membrane* is composed of a stratified scaly epithelium and a tunica propria beset with papillæ, that is built up of small, interlacing bundles of connective tissue and contains a few elastic fibers and a varying quantity of leucocytes. The latter occasionally exist in the form of solitary nodules; in this case numerous migrating leucocytes are found in the epithelium in these localities. The mucosa rests on a *submucosa*, which is composed of loosely-united connective-tissue bundles and robust elastic fibers. Glands are absent within the vaginal mucous membrane.

The *muscular coat* comprises an inner circular and an outer longitudinal layer of smooth muscle-fibers.

The outer *fibrous tunic* is a dense connective-tissue structure, rich in elastic fibers.

The *blood- and lymph-vessels* are arranged in parallel horizontal networks in the submucosa and in the tunica propria. Between the bundles of the muscular tunic lies a close network of wide venous channels. The *nerves* form a plexus in the outer fibrous tunic, beset with many small ganglia.

The mucous membrane of the *external genitalia* in the vicinity of the clitoris and the urethral orifice differs from the vaginal mucosa in the possession of numerous mucous glands, from 0.5 to 3 mm. in size, and on the labia minora in the presence of sebaceous follicles (without hair-follicles) from 0.2 to 2 mm. in size. The clitoris repeats on a diminutive scale the structure of the penis; end-bulbs and tactile corpuscles occur in the glans.

The *large glands of the vestibule* (Bartholin) are the homologues of the glands of Cowper in the male.

The labia majora are folds of the integument and possess the same structure.

The acid vaginal secretion contains desquamated scaly epithelial-cells and leucocytes, and not infrequently an infusorium, trichomonas vaginalis.

## TECHNIC.

No. 140.—For a general view of the testicle make a transverse incision * through the testicle and epididymis of a newborn child; fix the pieces in about 50 c.c. of Kleinenberg's picrosulphuric acid (p. 21) and harden in 30 c.c. of gradually-strengthened alcohol (p. 33). Stain thick transverse sections of the entire organ in dilute carmine (p. 36), and in Hansen's hematoxylin (p. 36), and mount in damar. Examine with very low magnification (Fig. 214). In the testicle of the rabbit, cat, and dog the corpus Highmori is not at the margin but in the center of the organ.

No. 141.—*Minute Structure of the Seminiferous Tubules.*—Place small pieces (2 cm. cubes) of the fresh testicle of an ox in 200 c.c. of Zenker's fluid (p. 31), and harden them in 50 c.c. of gradually-strengthened alcohols (p. 33). Cut sections as thin as possible, stain them in Hansen's hematoxylin (p. 36), and mount in damar (p. 45). Even with the low power tubules in a condition of activity can be distinguished from resting tubules; the former may be recognized by the intensely blue heads of the young spermatozoa (Fig. 215).

No. 142.—Still better preparations are obtained by placing the entire testicle of a mouse in 10 c.c. of the platinum-acetic-osmic acid mixture (p. 33) for twenty-four hours for fixation, then washing it for several hours in running water and placing it in 20 c.c. of gradually-strengthened alcohols for hardening. Mount the unstained sections in damar (Fig. 216). The platinum-acetic-osmium mixture does not penetrate sufficiently into the testicles of larger animals, which therefore are not suitable.

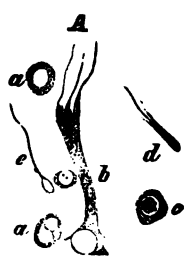



FIG. 237.—ISOLATED ELEMENTS OF THE TESTICLE OF AN OX. × 240. *a*, *c*, Mother-cells; *b*, "spermatoblast." *d*, Immature seminal filament; *e*, mature seminal filament.

No. 143.—*Elements of the Testicle.*—Place pieces about 1 cm. in size of the fresh testicle of an ox in 20 c.c. of one-third alcohol (p. 20) and in five or six hours tease the tubules in a drop of the same alcohol. Stain under the cover-glass with picrocarmine and mount in dilute glycerol. Several preparations from different parts of the organ should be completed and then not infrequently the cells of Sertoli with attached spermatocytes, or the seminal filaments produced by them, will be obtained (Fig. 237, *b*).

No. 144.—*Elements of the Semen.*—Make an incision into a fresh epididymis † and place one drop of the milk-white fluid that exudes from the cut surface on a clean slide; add one drop of salt solution, apply a cover-glass, and examine with the high power. After a time let one drop of distilled water flow under the

* If no incision is made into the organ, it does not harden sufficiently, because the dense tunica albuginea retards the penetration of the fluids.

† For a view of the spiral fiber mentioned above (p. 293, remark), that can be seen only with very powerful immersion lenses, I recommend the seminal filaments of the rat; they are to be examined in water.

cover-glass; the movements of the spermatozoa soon cease; the heads of the majority of the seminal filaments then present their broad surface and the tail curves and forms a loop (Fig. 217, 3). Remnants of protoplasm still adhere to seminal filaments not fully matured. The spermatozoa may be preserved by allowing the semen diluted with water to dry on the slide; then apply a cover-glass and secure it with cement (p. 45). In examining such preparations, too much illumination gives rise to troublesome reflections.

No. 145.—The vitality of the seminal filaments has led to *investigations for forensic purposes.* For example, it may be a question as to whether spots occurring on a linen garment were produced by semen. Cut strips from 5 to 10 mm. long from the suspected spots, soak them for from five to ten minutes in a watch-glass containing distilled water, and tease a few fibers. With the high power (500 : 1) chiefly examine the edges of the isolated linen fibers, to which the seminal filaments if present are attached. Not infrequently the heads are broken off; they are recognized by their peculiar luster, their shape, and their (in man small) size.

No. 146.—*Seminal Filaments of the Frog.*—The male frog is recognized by a well-developed wart on the ball of the thumbs. Open the abdominal cavity; the testicles are a pair of oval bodies (similar to the kidneys of mammals) lying to either side of the vertebral column. Divide the organ by a transverse incision; dilute a drop of the fluid with a drop of salt solution. The seminal filaments are large, the head thin and elongated, the tail so delicate that at the first glance it may be overlooked. Immature filaments lie grouped in tufts.

No. 147.—*Epididymis, Ductus Deferens, and Seminal Vesicles.*—Pieces from 1 to 2 cm. in size are to be fixed in about 100 c.c. of Zenker's fluid and hardened in 60 c.c. of gradually-strengthened alcohol (p. 33). Stain the sections with Hansen's hematoxylin and mount in damar (Fig. 218, 219, 220).

No. 148.—The *prostate* and the different divisions of the male urethra are to be prepared in 2 or 3 cm. cubes like No. 147 (Fig. 221).

No. 149.—*The Ovary.*—The ovaries of small animals may be fixed in toto and those of larger animals with several incisions transverse to the long axis in 100 or 200 c.c. of Zenker's fluid (p. 31) and hardened in 100 c.c. of gradually-strengthened alcohol (p. 33). For a topographical view (Fig. 222) it is advisable to cut thick sections, because otherwise the contents of the follicles easily fall out. Not every section includes large follicles; it is often necessary to cut many sections, in order to strike a favorable place. Stain the sections with Hansen's hematoxylin (p. 36), or in bulk with borax-carmine (p. 37). Mount in damar (p. 45).

No. 150.—Fresh *ova* may be obtained as follows. Procure the fresh ovaries of a cow. The large Graafian follicles are transparent, pea-sized vesicles, which with the scissors may be easily shelled out in toto. Transfer the isolated follicle to a slide and prick it with a needle. The needle must be carefully thrust in on the side of the follicle lying against the slide, otherwise the liquor will spurt out and carry the ovum with it.

With the low power, and without placing a cover-glass on the preparation, search for the ovum, which surrounded by the cells of the cumulus ovigerus will be found in the escaping liquor folliculi (Fig. 226, *A*). Place two narrow strips of paper on either side of the ovum, carefully apply a cover-glass, and examine with the high power.

The beginner will sacrifice many a follicle before he succeeds in finding an ovum. Often the ovum does not escape when the follicle is pricked; it may then be found by teasing the follicle.

No. 151.—*Ova of the Frog.*—Place a small piece of the fresh ovary of a frog on a slide and prick all the large pigmented eggs, so that their contents escape. Place that which remains in a watch-glass with distilled water and wash it by moving it to and fro with needles. Place the watch-glass on a black background; the smaller, still unpigmented follicles can then be seen. Transfer the washed object to a clean slide, apply a cover-glass, and examine it. The ova have very large germinal vesicles; the germinal spot disappears early, and usually is not to be seen. On the other hand, a dark spot occurs in the vitellus, the "nucleus of the vitellus." Surrounding the ovum is a finely-striated membrane, the inner surface of which is covered with flat cells; this is the theca folliculi with the simple follicular epithelium.

No. 152.—*The Oviducts.*—Pieces 1 or 2 cm. long are to be fixed in 50 c.c. of 3 per cent. nitric acid and after five hours hardened in 60 c.c. of gradually-strengthened alcohol. Stain with Hansen's hematoxylin and mount in damar.

No. 153.—For topographical preparations of the *human uterus* the uteri of young individuals are suitable. According to its size, fix the whole uterus or pieces of it 2 cm. square in about 100 c.c. of Zenker's fluid (p. 31) and harden in 100 c.c. of gradually-strengthened alcohol (p. 33). Stain in Hansen's hematoxylin and in eosin (p. 37) and mount in damar (p. 45). (Fig. 228.) In such preparations the gland follicles are often very indistinct.* In the two-horned uteri of many animals the often greatly-convoluted gland-tubules can be more readily distinguished; the arrangement of the muscular strata is different, more regular than in the human organ.

No. 154.—For preparations of the human *uterine mucosa*, cut out pieces 1 cm. square and treat them after No. 153. Owing to the extreme tortuousness of the glands, sections contain only fragments of the follicles. The cilia can seldom be seen in fixed preparations.

No. 155.—The *placenta* is to be treated according to No. 154.† Before cutting sections the pieces must be embedded in celloidin or paraffin; in the latter case the sections must be fastened to the slide (see Microtome Technic, Preservation of Sections), in order that the innumerable branches of the villi, cut in every plane, do not fall out. The study of preparations of this kind is one of the most difficult tasks of the microscopist.

* Fig. 228 was sketched from an unstained preparation. The glands were not so distinct as they appear in the illustration.

† Fixation in absolute alcohol often yields very good results.

# X. THE SKIN AND ITS APPENDAGES.

The skin is principally composed of connective tissue, which however is nowhere exposed, but is protected by a continuous epithelial coat. The connective-tissue portion of the skin is called *corium*, *dermis*, or *true skin*, the epithelial portion, *epidermis* or *cuticle*. The appendages of the skin, the *nails* and the *hairs*, as well as the *glands* and the *hair-follicles* embedded within the corium, are products of the epidermis.

## THE SKIN.

The surface of the *corium* is marked by many fine furrows, which intersect and enclose rectangular or lozenge-shaped areas or run parallel between minute ridges. The lozenge-shaped areas may be seen on the surface of the greater part of the body, while the ridges are confined to the volar surface of the hand and the plantar surface of the foot. The areas and ridges are beset with numerous conical elevations, the *papillæ*, the number and size of which vary greatly in different regions of the body. The largest (up to 0.2 mm. high) and most numerous papillæ occur on the palm of the hand and on the sole of the foot; they are very slightly developed in the skin of the face.

The corium chiefly consists of interlacing connective-tissue bundles, mingled with elastic fibers, cells, and smooth muscle-fibers. In the superficial strata of the corium the connective-tissue bundles are delicate and are united in a closely-interwoven texture; in the deeper strata they are larger and intersecting at sharp angles form a coarse-meshed network. These differences have led to the recognition of two strata in the corium, a superficial stratum beset with papillæ, *stratum papillare*, and a deep stratum, *stratum reticulare*. There is no sharp demarcation between the two strata, the one gradually blending with the other (Fig. 238). The stratum reticulare is connected with an underlying network of loosely-united bundles of fibrous tissue, the wide meshes of which contain clusters of fat-cells; this is the *stratum subcutaneum*. The storing of much adipose tissue in the interfascicular spaces of this stratum leads to the formation of the *panniculus adiposus*. The tissue of the subcutaneous

stratum is firmly or loosely connected with the fibrous sheaths of the muscles (the fasciæ) or with the periosteum of the bones. The elastic

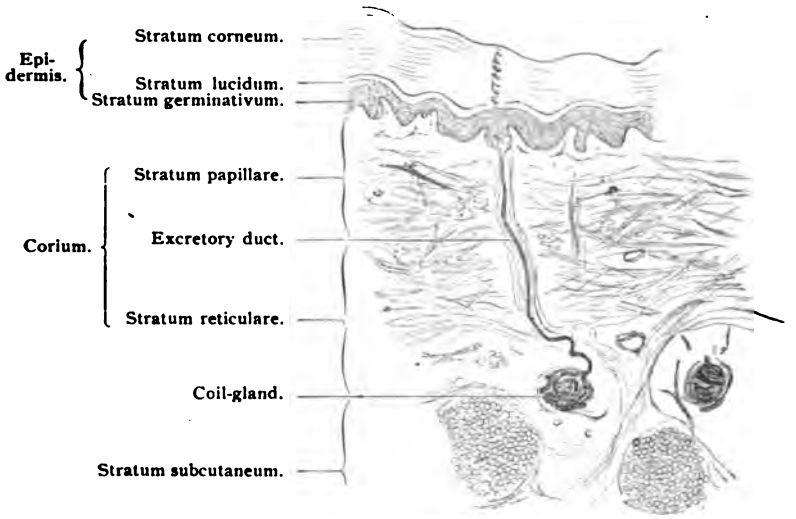



FIG. 238.—VERTICAL SECTION OF THE SKIN OF THE FINGER OF ADULT MAN. > 25. With this magnification the stratum granulosum is not visible. Techn. No. 156.

fibers, which are thin in the stratum papillare and thicker in the stratum reticulare, form networks uniformly distributed throughout the corium. The *cells* include spindle-shaped and plate-like connective-tissue elements,

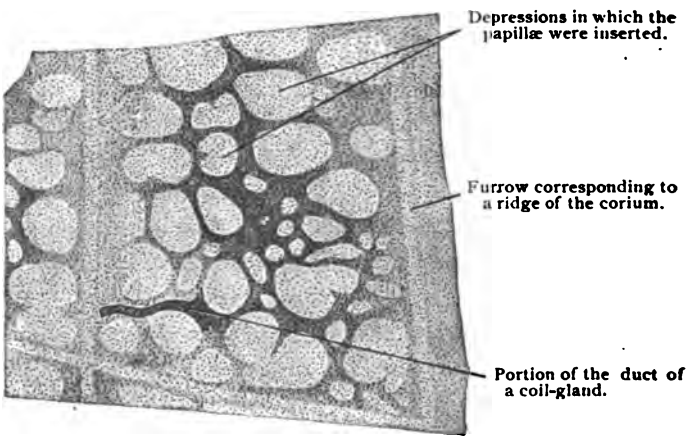



FIG. 239.—EPIDERMIS FROM THE SKIN OF THE DORSUM OF THE HUMAN FOOT, SEEN FROM THE LOWER SURFACE. × 120. The preparation is so to speak the cast, while the surface of the corium beset with papillæ represents the matrix. Techn. No. 157.

leucocytes, and fat-cells. The number of the cellular elements is extremely variable. The *muscle-fibers* almost exclusively belong to the

non-striped variety and the majority are attached to the hair-follicles; only in a few situations in the body do they occur spread out in the skin (tunica dartos, nipple). Striated muscle-fibers occur in the skin of the face, where they radiate from the mimetic muscles.

The *epidermis* consists of a stratified squamous epithelium, in which at least two sharply-defined zones may be distinguished: a deep zone, the *stratum germinativum* (Malpighi), which fills the depressions occurring between the papillæ of the corium, and a superficial, firmer zone, the *stratum corneum*. Both strata exclusively consist of epithelial-cells, which exhibit different appearances in the separate layers. In the deepest layer of the stratum germinativum the cells are cylindrical and possess oblong nuclei; these are followed by several layers of spherical cells that are beset with numerous minute thorns, the prickle-cells. The thorns are delicate thread-like processes, which penetrate the small amount of intercellular cement-substance occurring between the cells and unite neighboring cells to one another. Therefore they are called intercellular bridges (Fig. 14). In the stratum germinativum new cells are continually being formed by indirect division.

The *stratum corneum* is not everywhere of the same structure; two types may be distinguished: (1) In localities where the epidermis is well developed, as on the palm of the hand and the sole of the foot, a stratum of cells characterized by highly-refracting granules (keratohyalin granules) lies next to the stratum germinativum. The granules are produced by the cornification of some parts of the cell-protoplasm.* This stratum is named *stratum granulosum*. In the next layer the granules dissolve, blend with the parts of the protoplasm not yet transformed into horny substance, and form a uniformly clear zone, the *stratum lucidum*. This is covered by the broad *stratum corneum* proper. In this stratum all the noncornified parts of the cell under the influence of the atmosphere are desiccated; so it happens that each cell contains a delicate, horny mesh-work, and—since the intercellular bridges also become cornified—is enveloped in a horny membrane. The nucleus desiccates; the space which is occupied persists for a long period. These partly cornified, partly desiccated cells are only slightly flattened. (2) In situations where the epidermis is thinner, over the remaining surface of the skin, the stratum granulosum is narrow and interrupted. The stratum lucidum is absent. The horny cells of the stratum corneum are extremely flattened and are united in lamellæ. The last trace of the nucleus is lost.

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*These granules dissolve in a solution of potassium hydroxid and therefore are not composed of keratin, which is insoluble in this reagent.

The surface of the horny stratum undergoes a continual physiologic desquamation; the resulting loss is compensated by the pushing upward of the growing elements of the germinal stratum.

The color of the skin is due to the deposition of fine granules of pigment between and within the cells of the deeper layers of the epidermis; only in certain localities, for example, in the vicinity of the anus, are pigmented connective-tissue cells found in the adjacent corium.

With regard to the source of the pigment of the epidermis there are two theories, of which the one attributes its origin to the connective

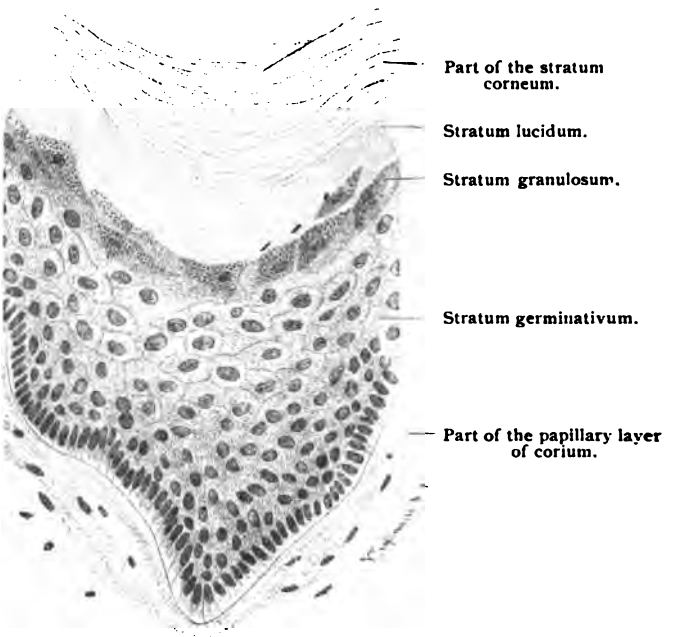


FIG. 240.—FROM A SECTION THROUGH THE SKIN OF THE SOLE OF THE FOOT OF ADULT MAN. × 360. Techn. No. 156.

tissue, the other to the epithelium. According to the first, hitherto frequently accepted opinion, the so-called "transportation" theory, the pigment is carried to the epithelium by pigmented connective-tissue cells, that wander from the corium into the epidermis and there disintegrate. In the human hair-bulb pigmented forms presenting great diversity in outline are found between the epithelial elements; some of these figures are cells, but it has not been demonstrated with certainty that they are connective-tissue cells, others are not cells, but intercellular clefts filled with pigment. The second theory is supported by the developmental history, which teaches that the pigment originates in the

epithelium of the hair without the intervention of connective-tissue cells. The pigment of the retina also is certainly and exclusively of epithelial origin.

## THE NAILS.

The nails are horny laminæ, which rest upon the *nail-bed*, a special modification of the skin. The nail-bed is bounded on the sides by the *nail-walls*, a pair of sloping folds with the descent forward. The nail-bed and nail-wall embrace a furrow, the *nail-groove*, in which the lateral borders of the nail are inserted (Fig. 241). The posterior border of the nail, the *nail-root*, rests in a similar but deeper groove, the *matrix*,* in which the principal growth of the nail takes place.

The anterior free border of the nail projects over the *nail-ridge*, a small seam-like prominence at the distal end of the nail-bed.

The *nail-bed* consists of corium and of epidermis. The fibro-elastic

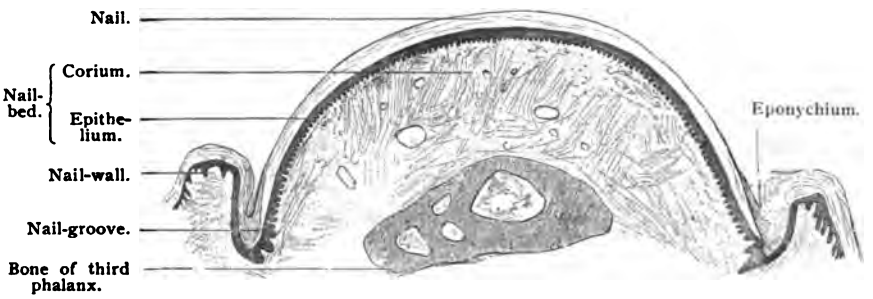


FIG. 241.—DORSAL HALF OF A CROSS-SECTION OF THE THIRD PHALANX OF A CHILD. × 15. The ridges of the nail-bed in cross-section appear like papillæ. Techn. No. 158.

bundles of the corium partly are disposed parallel to the long axis of the finger, partly run vertically from the periosteum of the phalanx to the surface. The surface of the corium does not possess papillæ, but minute longitudinal ridges. They begin low at the matrix, increase in height toward the anterior border of the nail, and terminate abruptly at the point where the latter leaves its bed. The epithelium is of the stratified scaly variety, of the same structure as that of the germinal stratum of the epidermis. It covers the ridges of the nail-bed, fills up the furrows between them, and is sharply defined from the substance of the nail. The matrix, likewise, consists of corium and epidermis; the corium is distinguished by its tall papillæ, the stratified scaly epithelium is very thick and is not sharply defined from the nail-substance, but gradually blends

* Some authors name the whole nail-bed matrix, which is in a measure justified by the growth in thickness of the nail that occurs here.

with the latter. This is the place where by continual division of the epithelial-cells the material for the growth of the nail is furnished. On this account the epithelium is called the *germ-layer* of the nail. The extent of the matrix is indicated by the *lunula*, a white anteriorly-convex area, visible to the unaided eye; it is produced by the thick, uniformly-extended germ-layer. The *nail-wall* exhibits the usual structure of the skin. The germinal stratum of the nail-wall gradually blends with the epithelium of the nail-bed; the horny stratum extends into the nail-groove and as "eponychium" covers a small portion of the border of the nail, but soon diminishes in thickness and disappears (Fig. 241).

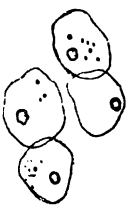

FIG. 242.—ELEMENTS OF HUMAN NAIL. × 240. Techn. No. 158 *a*.

The *nail* itself consists of horny epithelial scales, that are very firmly united with one another and are distinguished from the horny cells of the stratum corneum of the epidermis by the possession of a nucleus (Fig. 242).*

## THE HAIRS AND THE HAIR-FOLLICLES.

The hairs are flexible, elastic, horny threads, which are distributed over nearly the entire surface of the body and on the integument of the cranium are united in small groups. The part of the hair which projects beyond the free surface of the skin is called the *shaft;* the portion obliquely embedded within the integument, the *root;* this at its lower extremity is expanded to a hollow knob, the *hair-bulb*, which is occupied by a formation of the corium, the *hair papilla* (Fig. 243).

Each hair-root is inserted in the *hair-follicle*, a modification of the skin in the formation of which corium and epidermis participate; the parts furnished by the latter are named the *epithelial root-sheaths*, the portion originating from the corium, the *dermal* or *connective-tissue sheath*. From two to five glands, the *sebaceous glands*, open laterally into the upper part of the follicle. Bundles of smooth muscle-fibers, the *arrectores pilorum*, pass obliquely from the upper surface of the corium and attach themselves beneath a sebaceous gland to the fibrous sheath of the hair-follicle; the point of insertion of these fibers is always on the side toward which the hair inclines and forms an acute angle with the free surface of the skin; consequently when they contract, the follicle and the shaft become erect.

* The new anatomic nomenclature reckons the epithelium of the nail-bed to the nail, that according to this representation consists of stratum corneum and stratum germinativum.

The hair consists entirely of epithelial-cells, which are arranged in three well-defined strata: (1) the *cuticle*, which covers the surface of the hair; (2) the *cortical substance*, which forms the chief bulk of the hair; (3) the *medulla*, which occupies the axis of the hair.

The *cuticle* consists of transparent imbricated scales: horny epithelial-cells without nuclei.

The *cortical substance* of the shaft consists of elongated horny epithelial-cells with attenuated nuclei, which are very intimately united with one another; on the root the cells become the softer and rounder, their nucleus correspondingly the more spherical, as they approach the hair-bulb.

The *medulla* is absent in many hairs; when it is present (in the

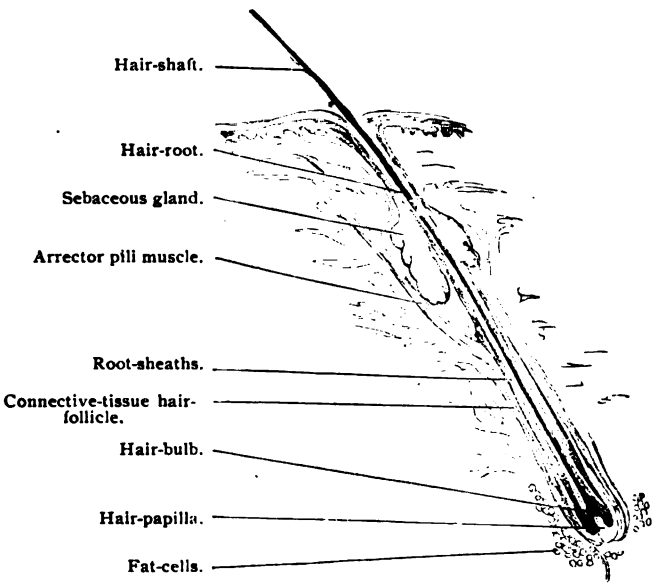


FIG. 243.—FROM A THICK CROSS-SECTION OF HUMAN SCALP. × 20. Techn. No. 161.

thicker hairs) it does not extend through the entire length of the hair. It consists of cubical, finely-granular epithelial-cells, which contain a rudimentary nucleus and are usually disposed in twofold rows.

The colored hairs contain pigment, diffused, and in the form of granules, which partly occur between and partly within the cells of the cortical substance.* In every hair which has attained its full development extremely minute *air-vesicles* occur; they are found in the cortical substance as well as in the medulla, and also in the intercellular clefts.

* As to the source of the pigment, see page 324.

The *follicle* of finer (lanugo) hairs is formed alone by the epidermal root-sheaths, but in coarser hairs the corium participates in its construction. In the follicles of the latter the following strata may be distinguished: an *outer longitudinal stratum* formed of loosely-united, longitudinally-disposed bundles of connective tissue, mingled with elastic fibers and richly supplied with blood-vessels and nerves; next follows a *middle circular stratum*, thicker, consisting of small fibrous bundles circularly arranged, which is contiguous to an *inner* clear, homogeneous belt, the *glassy* or *hyaline membrane*, resembling in character the elastic membranes. Elastic fibers do not occur in the middle layer nor in the papilla. These

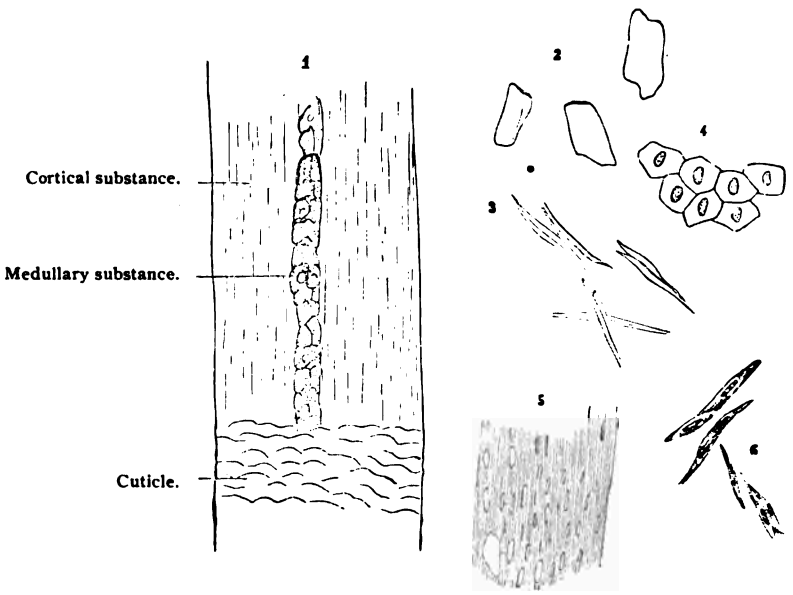


FIG. 244.—ELEMENTS OF A HUMAN HAIR AND HAIR-FOLLICLE. × 240. 1, White hair; 2, scales of the cuticle; 3, cells of the cortical substance of the shaft; 4, cells of Huxley's layer; 5, cells of Henle's layer having the appearance of a fenestrated membrane; 6, cells of the cortical substance of the root. Techn. No. 159 *a* and No. 160.

three strata are derived from the corium and together named the *dermal* or connective-tissue hair-follicle. Within the hyaline membrane lies the *outer root-sheath*, which as a continuation of the germ-layer of the epidermis consists of stratified scaly epithelium; inward to this lie continuations of the stratum granulosum and stratum corneum, which extend about to the point where the ducts of the sebaceous glands open into the follicle; immediately below (toward the papilla) the *inner root-sheath* begins *abruptly*, which in the lower portion of the follicle is differentiated into two sharply-defined layers. The outer of these two, *Henle's layer*, consists of a single or double row of epithelial-cells

without nuclei (here and there an atrophic nucleus is present), while the inner, *Huxley's layer*, is formed of a simple stratum of nucleated cells. The inner surface of this layer is lined by a delicate membrane, the *cuticle of the root-sheath*, which exhibits the same structure as the cuticle of the hair. Toward the base of the follicle the outer root-sheath diminishes in thickness and disappears; the elements of the inner root-

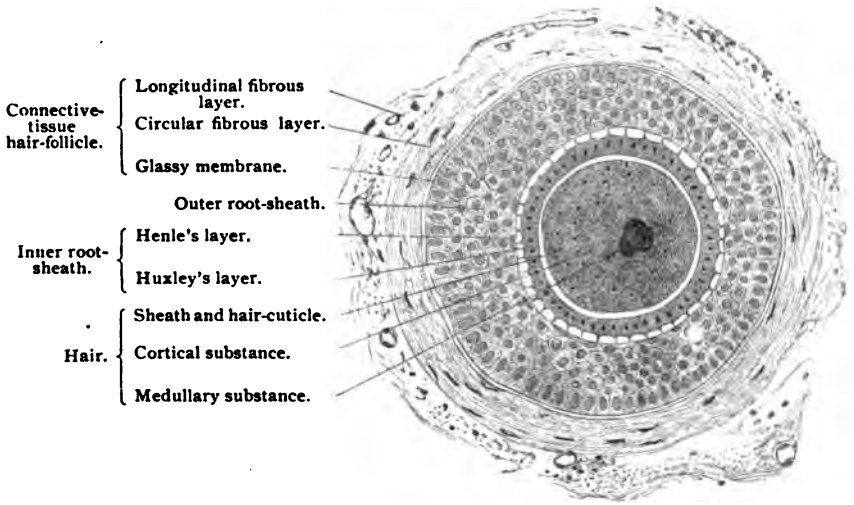


FIG. 245.—FROM A HORIZONTAL SECTION OF HUMAN SCALP. × 240. Cross-section of a hair and hair-follicle in the lower half of the root. Techn. No. 161.

sheath, as well as those of the cuticulæ, all become nucleated cells, that can be distinguished as separate layers until near the neck of the papilla; there they lose their sharp demarcation and gradually coalesce with one another, but nevertheless can be distinguished from the cells of the hair-bulb by the pigmentation of the latter.*

## DEVELOPMENT OF THE HAIR.

The first anlage of the hair and of the hair-follicle appears at the end of the third embryonal month, in the form of a local thickening of the epidermis, which is chiefly effected by elongation of the (columnar) cells of the deepest layer of the germinal stratum. This thickening grows in length down into the corium (Fig. 246, *A*) and forms a solid epidermal peg, the *hair-germ* (Fig. 246, *A*, *B*), that at its lower end

* Already at the level of the papilla keratohyalin granules appear in the cells of Henle's layer, at a somewhat higher level also in those of Huxley's layer, that a little farther up disappear; from this upwards the elements of the inner root-sheath are corneous.

becomes expanded and club-shaped (Fig. 246, *C*). Meanwhile the papilla (*C*, *p*) and the dermal portion of the hair-follicle (*C*, *hb*) develop by differentiation of the connective tissue of the surrounding corium. The hair-germ separates into an outer stratum and into an inner axial cord (*D*, *s*). The former becomes the outer root-sheath (*aw*), the

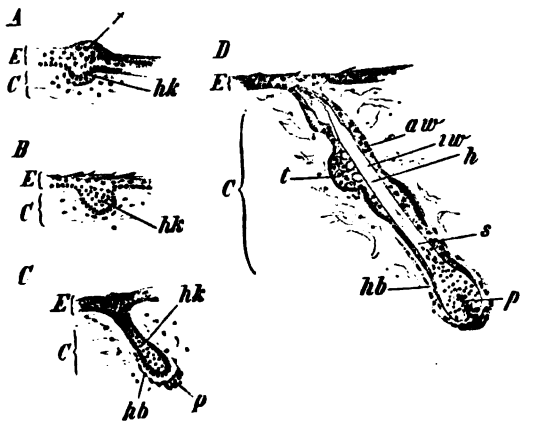


FIG. 246.—FROM A VERTICAL SECTION (*A*) OF THE SKIN OF THE CHEEK OF A FOUR MONTHS' HUMAN EMBRYO AND (*B*, *C*, *D*) OF THE SKIN OF THE FOREHEAD OF A HUMAN EMBRYO FIVE AND A HALF MONTHS OLD. × 80. *E*. Epidermis, consisting throughout of nucleated epithelial-cells; *C*, corium; *x*, thickening; *hk*, hair-germ; *hb*, connective-tissue hair-follicle; *p*, papilla; *aw*, outer root-sheath; *s*, axial portion, in which in the upper division the separation into (*iw*) inner root-sheath and (*h*) hair is visible; *t*, anlage of the sebaceous glands. Techn. No. 162.

peripheral portion of the axial strand becomes the inner root-sheath (*iw*), the central part, the hair (*h*). The sebaceous glands arise as local outgrowths of the outer root-sheath (*t*).

The development of hairs in the manner described may occur after birth and until late in life.

### GROWTH OF THE HAIR AND OF THE ROOT-SHEATHS.

The growth of the hair, of the cuticular sheaths, and of the inner root-sheath takes place by continual mitotic division of the epithelial elements around the papilla, that becoming horny annex themselves from below to previously cornified cells. Therefore the tip is the oldest, the portion lying immediately above the hair-bulb the youngest, part of the hair. The outer root-sheath, on the other hand, grows in a radial direction from the inner surface of the glassy membrane towards the axis of the hair.

### SHEDDING AND REPLACEMENT OF HAIR.

After birth all the hairs are shed and replaced by others. In the adult a constant, but not periodic, replacement of the dead hairs of the

scalp and beard occurs. (With regard to the shedding of the other hairs nothing is definitely known.)

The minute details of the process are as follows: the hair-bulb becomes horny and frayed, like a brush; the now dead hair pushes upward from the papilla,* the empty root-sheaths collapse, while at their inferior extremity lies the papilla, atrophied and altered in form (Fig. 247). After a (often long) period the epithelial elements of the empty

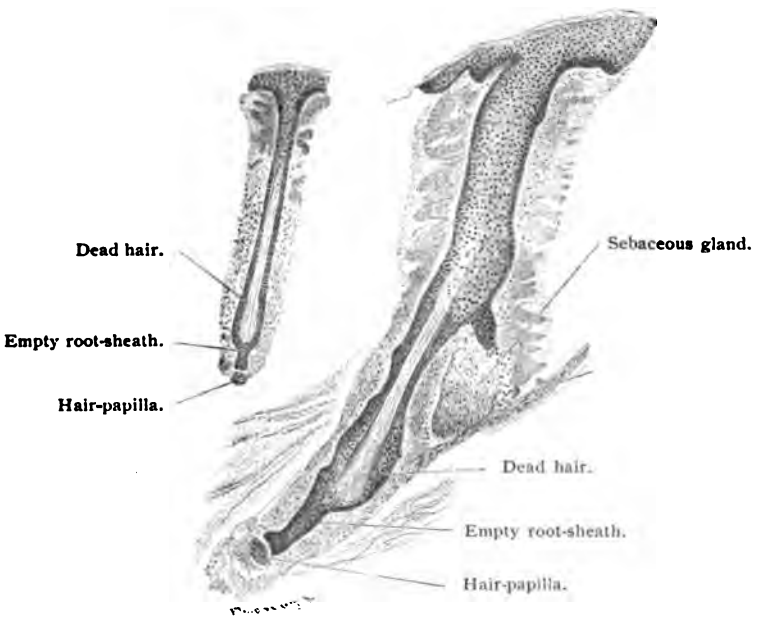


FIG. 247.—FROM A VERTICAL SECTION OF THE HAIRY SCALP OF ADULT MAN. × 40. Techn. No. 163.

root-sheaths begin to grow and form a new hair-germ, from which the new hair develops by the same processes as the embryonal hair. The new hair thus formed pushes itself upward under and beside the effete hair, while the latter after a shorter or longer period falls out.

## THE GLANDS OF THE SKIN.

The *sebaceous glands* are either unbranched or branched simple saccular glands. Each gland consists of a short excretory duct (Fig. 248, *A*, *a*) and of a variable number of little gland-sacs (*t*). The *duct* is lined by stratified scaly epithelium, an extension of the outer root-

* Further growth of the cornified hair does not occur; the ascent is passive and dependent on the multiplication of the non-cornified epithelial-cells lying under the dead hair.

sheath, which by a gradual decrease in the number of its layers passes into the epithelial lining of the gland-sacs. This at the beginning consists of low cuboidal cells (Fig. 248, *B*), that toward the interior are followed by spherical or polyhedral elements varying in size, which fill the entire gland-sac and exhibit all the transitional phases in the process by which the cell is converted into the secretory product of the gland (Fig. 248, 2, 3, 4). The secretion, the *sebum*, during life is a semifluid substance that consists of fat and disintegrated cells. While the sebaceous glands occur as appendages of the hair-follicles of the coarser hairs (Fig. 243), in the case of the lanugo hairs reversed relations prevail, since the follicles of the latter appear as the appendages of the powerfully-developed sebaceous glands (Fig. 248, *A*). The sebaceous

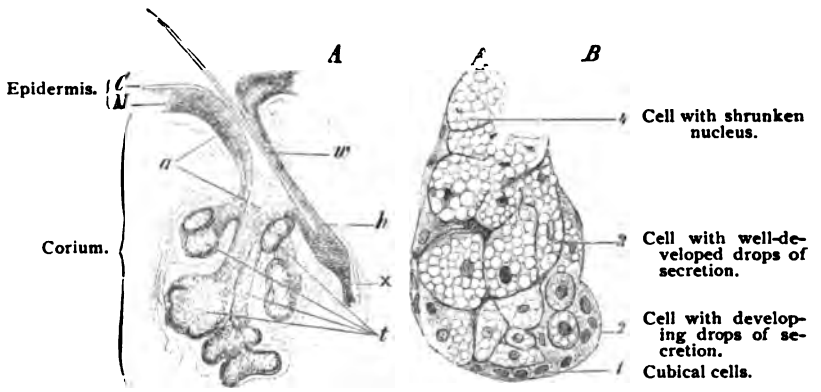


FIG. 248.—*A*. FROM A VERTICAL SECTION THROUGH THE ALA NASI OF A CHILD. × 40. *C*. Stratum corneum; *M*, stratum germinativum; *t*, sebaceous gland consisting of four follicles, *a*, duct of the same; *w*, lanugo hair, about to be shed, *h*, hair-follicle of the same, at the base of which a new hair, *x*, is forming.

*B*. FROM A VERTICAL SECTION OF THE SKIN OF THE ALA NASI OF AN INFANT. × 240. Follicles of a sebaceous gland containing gland-cells in various stages of secretion. Techn. No. 164.

glands are distributed with the hairs over the entire body and are wanting only where they are absent, on the palm of the hand and on the sole of the foot. There also are sebaceous glands that are not associated with hair-follicles; for example, on the red edge of the lips, on the labia minora, on the glans, on the prepuce of the penis; in the latter situation they are known as *glandulæ præputiales* (Tyson). The sebaceous glands are always situated in the superficial layers of the corium, in the stratum papillare. Their size varies from 0.2 to 2.2 mm.; the latter are found in the integument of the nose, where their excretory ducts are visible to the unaided eye.

The *coil-glands* (sudoriparous or sweat-glands) are long, unbranched tubules, that at their lower ends are rolled into a spherical coil having a

diameter of 0.3 to 7 mm. (of the latter size in the axilla). Two parts are distinguished, the *excretory duct* and the *coil* (Fig. 238). The *duct* runs a straight or sinuous course through the corium, enters the epidermis between two papillæ, through the stratum corneum of which it is spirally twisted, and opens on the surface of the skin by a rounded orifice, the *sweat-pore*, just visible to the naked eye. The walls of the duct consist of longitudinally-disposed bundles of connective tissue, lined within by several layers of cubical epithelial-cells. The *coil* is a greatly-convoluted simple canal, the walls of which are formed of a simple layer of cubical cells, containing granules of pigment and of fat, surrounded by a delicate membrana propria. In well-developed glands longitudinally-disposed smooth muscle-fibers occur between the membrana propria and the gland-cells. Branched tubules have been observed only in the axillary and circumanal glands.

The secretion usually is an oily fluid substance, for the purpose of lubricating the skin; only under the influence of disturbed innervation do the coil-glands discharge the watery liquid called sweat. The coil-glands are distributed over the entire surface of the skin and are absent only on the glans and on the inner surface of the prepuce. They are most numerous in the skin of the palm of the hand and of the sole of the foot.

## THE BLOOD-VESSELS, LYMPH-VESSELS, AND NERVES OF THE SKIN.

The *arteries* of the skin originate in a network lying above the fasciæ and branch as they pass toward the surface of the skin. These branches anastomose with one another and with those of neighboring arteries and in the lower stratum of the corium form a horizontally-disposed network, the *cutaneous network*. The arteries supplying the skin are therefore not end-arteries.*

From this network two capillary territories are supplied; the deeper is intended for the adipose tissue (Fig. 249, *a'*), the more superficial appears in the form of basket-like plexuses surrounding the coil-glands (*a''*). From the cutaneous network twigs ascend that anastomose and form a second horizontal network in the upper third of the corium, the *subpapillary plexus;* from this very small twigs arise, which run for a short distance along the rows of papillæ and send little branches into

* "End-arteries" are those small arteries which do not anastomose with neighboring arteries, but independently supply capillary circuits of varying extent. When they become obstructed the part of the organ which they supply dies.

them (Fig. 249, $a'''$). These smallest twigs do not anastomose with one another, hence are end-arteries. The branches for the hair-follicles and sebaceous glands also arise from the subpapillary plexus.

The blood returning from the capillaries of the papillæ, the hair-follicles, and the sebaceous glands is taken up by *veins* that form a dense horizontal plexus lying beneath the papillæ and that occasionally are united with a second horizontal plexus lying close below the first. From this plexus small venous trunks descend beside the arteries and lead to a third network lying in the lower half of the corium, which is not so

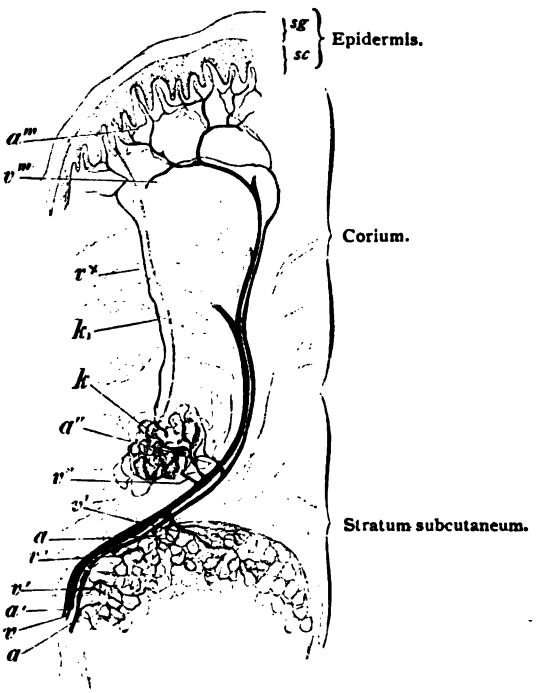


FIG. 249.—FROM A VERTICAL SECTION OF THE SKIN OF THE SOLE OF A HUMAN FOOT. × 50. *sc*, Stratum corneum; *sg*, stratum germinativum; *a*, artery; *v*, vein; *a' v'*, branches to the panniculus adiposus; *a'' v''*, branches to the coil-glands; *k*, duct of the same; *vx*, vein accompanying the duct. Techn. No. 165.

evenly spread out as those preceding. This plexus takes up the veins coming from the coil-glands and then those proceeding from the lobules of adipose tissue. It should further be noted that a branch of the veins of the coil-glands passes along the excretory duct to the venous plexus of the stratum papillare (Fig. 249, *v* x) and that the hair-papilla receives an independent arterial branch. From the third venous network larger veins lead to the lower boundary of the skin, where a fourth horizontally-disposed, "subcutaneous" venous network occurs, from which still

larger trunks turn into the subcutaneous tissue and then unite with the large subcutaneous veins, some of which are provided with names. The *lymph-vessels* form two horizontal capillary networks, of which that consisting of smaller channels and narrower meshes lies in the papillary stratum of the corium beneath the vascular network; the other, wider-meshed, is situated in the subcutaneous tissue. Special networks of lymph-capillaries surround the hair-follicles, the sebaceous and coil-glands.

The *nerves* of the integument (numerous in the palm of the hand and the sole of the foot) partly terminate in the subcutaneous tissue in lamellar corpuscles; partly they end in tactile-corpuscles, in tactile-cells, and as intra-epithelial fibrils (Fig. 122). The hairs are also supplied with medullated nerve-fibers, which run up to the point where the sebaceous glands open into hair-follicles; here they divide, lose their medullary sheath, and as naked axis-cylinders, usually running longitudinally, terminate in a spoon-shaped expansion *on* the glassy membrane (epilemmal nerve-ending); in the tactile-hairs (sensory hairs) of animals delicate twigs arise from these nerves, which pass through the hyaline membrane of the hair-follicle into the outer root-sheath and there end in tactile-discs. The hair-papilla does not possess nerves. In regard to the nerves of the coil-glands, see page 243.

## THE MAMMARY GLANDS.

The mammary gland consists of from fifteen to twenty tubular glands, which are held together and united in a common body by loose connective tissue containing fat-cells. Each of these glands has its own excretory duct opening on the nipple, that shortly before its termination is provided with a conspicuous spindle-shaped expansion, the *ampulla* or *sinus lactiferus*, and by means of dichotomous ramifications is connected with the terminal compartments. The latter lie close together and are united by connective tissue into small lobules.

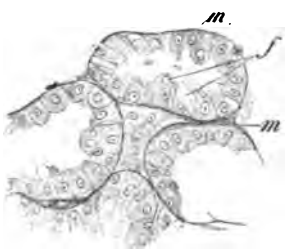


FIG. 250.—FROM A THIN CROSS-SECTION OF THE MAMMARY GLAND OF A PREGNANT RABBIT. × 240. *f*, Fat in the gland-cells; *m*, membrana propria. Techn. No. 167.

The excretory ducts are composed of a columnar epithelium, which in the larger branches not infrequently is replaced by a stratified scaly epithelium, surrounded by a membrana propria, outside of which are fibrous bundles which are in general circularly disposed. The gland-tubules are lined by a simple layer of epithelial-cells the height of which varies greatly; they are low

when the tubules are filled, cubical or columnar when the tubules are empty. In the latter case the cells contain globules of fat. The glandular elements rest upon a membrana propria composed of cells (p. 81), which is enveloped by loose connective tissue intermingled with varying numbers of leucocytes and plasma-cells.

After lactation is ended a gradual regressive metamorphosis occurs, that is indicated by abundant development of the interlobular connective tissue. The lobules become smaller, the tubules begin to atrophy. In elderly persons all the lobules and tubules disappear and only the excretory ducts remain.

In children of both sexes the mammary gland chiefly consists of connective-tissue, within which the branched excretory ducts, their ends terminating in a bulbous enlargement, are enclosed. Tubules are

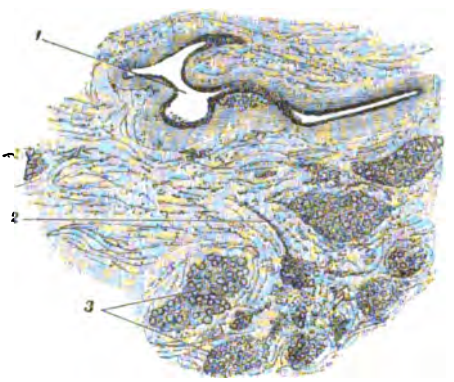


FIG. 251.—FROM A THICK SECTION OF THE MAMMARY GLAND OF A WOMAN LAST PREGNANT TWO YEARS BEFORE. × 50. 1. Large excretory duct; 2, small excretory duct; 3, gland-lobules, separated from one another by connective tissue. Techn. No. 166.

wanting. The mammary gland of the adult male exhibits the same structure.

In the adult female before pregnancy has occurred the mammæ are disc-shaped bodies, that principally consist of connective tissue and of the excretory ducts. Only a few gland-tubules at the extremites of the smallest branches of the ducts are present.

The integument of the *nipple* and of the *areola* is characterized by deep pigmentation, by tall papillæ, and by the presence of smooth muscle-fibers, which latter partly are circularly arranged around the orifices of the galactophorous ducts, partly vertically to the apex of the nipple. The pigmentation is due to the presence of pigment-granules in the deepest layers of the epidermis. In the integument of the areola accessory mammary glands, the *areolar glands* (Montgomery) occur.

The *blood-vessels* approach the mammæ from all sides and form capillary networks embracing the gland-tubules. The *lymph-vessels* form capillary plexuses lying within and between the gland-lobules. Lymphatic networks also occur in the vicinity of the ampullæ and the areolæ.

The nerves are in part distributed to the blood-vessels, in part behave like those of the salivary glands (p. 243).

Microscopically *milk* consists of a clear fluid, the *milk plasma*, in which *milk-globules*, from 2 to 5 $\mu$ in size, are suspended. Owing to the fact that the globules do not coalesce, the presence of a delicate membrane of casein is assumed. In addition, isolated cells enclosing oil-globules (leucocytes?) are found in milk.

The elements of the milk secreted before and in the first few days after parturition include, beside the milk-globules, the so-called *colostrum-corpuscles*, nucleated cells, some of which contain minute yellow-colored and larger uncolored fat-droplets, others only uncolored fat-droplets.

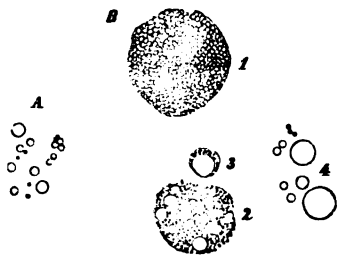


FIG. 252.—*A*. MILK-GLOBULES FROM HUMAN MILK. × 560. Techn. No. 168. *B*. ELEMENTS OF THE COLOSTRUM OF A PREGNANT WOMAN. × 560. 1. Cell containing uncolored fat-globules; 2, cell containing minute colored fat-globules; 3, leucocyte; 4, milk-globules. Techn. No. 168 *a*.

The mode in which the glandular epithelium participates in the formation of the milk-globules and the colostrum-corpuscles is not yet altogether clear. Only this much is known with certainty, that the cells do not perish in the act of secretion. It is a question whether the fat contained within the glandular cells is discharged alone or with the portion of the cell directed toward the lumen of the tubule.

## TECHNIC.

No. 156.—*Strata of the Skin; Coil-glands.*—Cut from the pad of the finger, the palm of the hand, or the sole of the foot pieces of skin, as fresh as possible, from 1 to 2 cm. square together with a thin stratum of the subjacent fat and place them in 30 c.c. of absolute alcohol. To prevent curling of the pieces pin them on a small cork-plate with the epidermis turned toward the cork, and place the whole in absolute alcohol. On the following day remove the pieces from the cork-plate and place them for from three to four weeks in 50 c.c. of 90 per cent. alcohol. Cut thin and thick sections. The latter are indispensable in order to see the excretory ducts of the coil-glands in their entire length. The most suitable for this purpose is the skin of the sole of the foot of children, because the still short ducts of the coil-glands here run vertically (Fig. 238). Stain with alum carmine, ten minutes (p. 36); the red coils can

be seen with the unaided eye; mount in damar. Examine with the low power. In thick sections the papillæ often are indistinct, because they are encircled by the red-colored stratum germinativum, the screw-like twisted ends of the excretory ducts may be most distinctly seen when the object is faintly illuminated or with oblique illumination (see p. 50, remark *).

To render the stratum granulosum visible, bulk-staining with borax-carmine for two or three days (p. 37), is to be recommended. The granules of this stratum are then stained an intense red (Fig. 240).

No. 157.—Pretty preparations of the under surface of the *epidermis* are obtained by fixation of shreds of the epidermis of the dorsum of the foot, that can often be detached from injected cadavers, in 30 c.c. of absolute alcohol. Stain for two minutes in Hansen's hematoxylin and mount in damar (Fig. 239).

No. 158.—For preparations of the *nails* fix the distal phalanx of a child from eight to twelve years of age (in adults, that of the little finger, if possible of women), two or four weeks in 100 or 200 c.c. of Müller's fluid and harden in about 100 c.c. of gradually-strengthened alcohol; decalcify (p. 33); harden again and stain thick cross-sections ten minutes in alum-carmine (p. 36). (Fig. 241.) In cutting sections place the knife on the volar side (not on the nail side) of the phalanx. The substance of the nail frequently shows differently-colored strata. In the nails of old cadavers the epithelium often becomes loosened from the ridges.

No. 158 *a*.—*Elements of the Nails.*—Place pieces of nail 1 or 2 mm. broad in a test-tube containing 5 c.c. of concentrated potash-lye and heat it over a flame until it boils up once. Transfer the nail with a drop of the lye to a slide and scrape off some of the softened surface; apply a cover-glass. On examination with a high power, cells will be found like those in Fig. 242. For comparison, investigate the horny cells of the stratum corneum, which may be obtained by lightly scraping the pad of the finger with the handle of a scalpel. Examine the polygonal scales in a drop of distilled water, with a high power.

No. 159.—*Hairs.*—Place a hair in a drop of salt solution on a slide and examine it with the low and the high power; the most suitable for study are white hairs and the hairs of the beard. The hair cuticle of man is very delicate and the transverse markings produced by the imbrication of the cells are often very indistinct; usually only fine wavy lines are visible. The hairs of many animals, on the other hand, show the cuticula very well, for example, sheep's wool.

No. 159 *a*.—For the demonstration of the *elements of the hairs*, place a piece of hair 1 or 2 cm. long in a drop of pure sulphuric acid on a slide and apply a cover-glass; press lightly on the cover-glass with a needle and the cortical substance will split up into fibers, which consist of adherent cortical cells. Slightly warm the slide, press again with a needle, so that the cover-glass becomes slightly displaced; numerous free elements, superficial scales and cortical cells, will then be seen.

No. 160.—For the exhibition of the *elements of the hair-follicles* (and the *hairs*) cut from a mustachioed human upper lip a piece 2 cm. square and place it in dilute acetic acid (5 c.c. of acetic acid to 100 c.c. of distilled water). In two days the individual hairs with their sheaths can be easily withdrawn and their elements separated by teasing in a drop of distilled water (Fig. 244). The cells of Henle's sheath float in small complexes in the preparation and closely resemble fenestrated membranes (Fig. 244, 5). The fenestra are spaces normally occurring between Henle's cells, through which processes of the cells of Huxley's stratum extend to the outer root-sheath. Not infrequently a hair-follicle is obtained at the base of which a new hair is developing (similar to Fig. 247).

No. 161.—*For the study of hairs and hair-follicles* place pieces 2 or 3 cm. square of the fresh skin of the scalp in about 200 c.c. of a 2.5 per cent. solution of potassium bichromate (p. 21, 9) for from four to eight weeks; wash them from one to three hours in running water and harden in the dark in about 100 c.c. of gradually-strengthened alcohol. Longitudinal sections which include the entire length of the follicle are very difficult to cut. Macroscopic orientation as to the direction of the hair is first necessary. To obtain preparations like that in Fig. 243 thick sections, unstained, are to be mounted in glycerol. Thin sections usually include only a portion of the hair-follicle. It is much easier to cut thin cross-sections, but care must be taken to make the cut *vertical to the longitudinal direction of the hair*, not parallel to the surface of the skin. In this way a *single* section shows different levels of hairs and hair-follicles; such sections are to be stained in dilute carmine (p. 36), and Hansen's hematoxylin, or better, first with hematoxylin and then with picrocarmine (p. 36) ten minutes, and mounted in damar. Especially instructive are the sections through the hair-follicle close to the hair-bulb (Fig. 245).

No. 162.—*For the development of hair* cut pieces about 2 cm. square of the skin of the forehead (not of the hairy scalp) of a five- or six-months'-old human embryo; span them out (see No. 156); place them for fourteen days in 100 or 200 c.c. of Müller's fluid and harden in about 100 c.c. of gradually-strengthened alcohol. Stain the tissue in bulk in borax-carmine (p. 37). The sections may also be stained in Hansen's hematoxylin (p. 36). Embed the tissue in liver; endeavor to cut sections exactly in the direction of the hair-follicle, which is much more easily done than in the hairy scalp of the adult. Mount in damar. The sections exhibit all stages of development (Fig. 246). The epithelial thickenings are only to be seen in well-preserved epidermis, which in embryos is often somewhat macerated. They are more easily found in embryos of the lower animals.

No. 163.—*Shedding and Replacement of Hair.*—The eyelids of newborn children are most suitable. Treat like No. 184. Cut sagittal sections. Vertical sections of the hairy scalp often yield good results (Fig. 247).

No. 164.—*The Sebaceous Glands.*—Fix and harden the alæ nasi of newborn children in 100 c.c. of a 2.5 per cent. solution of potassium bichromate (like No. 161). Cut thick and thin sections; stain them with dilute carmine (p. 36), and with Hansen's hematoxylin (p. 36), and mount in damar. Sections lengthwise to the dorsum of the nose often show sebaceous glands and hair-follicles, but they must be exactly vertical (Fig. 248). The alæ of the nose of adults, on account of the very large sebaceous glands with their wide excretory ducts, do not furnish good microscopic specimens. Small sebaceous glands with hair-follicles can be seen with the unaided eye in stripping off the macerated epidermis of old cadavers.

No. 165.—*Blood-vessels of the Skin.*—Inject with Berlin blue the entire hand of a child through the ulnar artery (or a foot through the posterior tibial artery) and place it in from 1 to 2 liters of Müller's fluid; after several days cut pieces 2 or 3 cm. square of the palm of the hand or of the sole of the foot, place them (two or four weeks) in 100 or 200 c.c. of Müller's fluid and harden them in 100 c.c. of gradually-strengthened alcohol. Cut thick sections and mount them unstained in damar (Fig. 249). The papillæ in such sections can only be recognized by the capillary loops. To the beginner it appears as if the loops extend into the stratum germinativum.

No. 166.—*For a general view of the mammary gland* place the nipple and a portion of the gland (3 or 4 cm. square) in 60 or 100 c.c. of absolute alcohol. If possible, obtain the glands of an individual that was pregnant not too long a time before, also the glands of virgins, etc. Make vertical sections through the nipple and in any direction through the gland-substance, and stain them with Hansen's hematoxylin; mount in damar (Fig. 251).

No. 167.—*For the minute structure of the mammary glands* place the warm living tissue (3 or 5 mm. cubes) of a pregnant mammal in 5 c.c. of Flemming's mixture (p. 32), and harden after one or two days in 30 c.c. of gradually-strengthened alcohol. Cut very thin sections, stain them with safranin (p. 38), and mount in damar (Fig. 250). The structure is often difficult to understand on account of the small size of the gland-cells (in the rabbit).

No. 168.—*Elements of Milk.*—Put a drop of salt solution on a clean slide and add to it a drop of milk. The milk is to be obtained by placing the cover-glass upon the nipple and then pressing out a drop. Examine with a high power (Fig. 252, *A*).

No. 168 *a.*—*Elements of Colostrum.*—Obtain the colostrum shortly before parturition. Proceed as in No. 168. Be careful to avoid pressure on cover-glass. The nuclei of the colostrum corpuscles can rarely be distinctly seen without further treatment; on the addition of a drop of picrocarmine they appear as dull-red spots (Fig. 252, *B*).

# XI. THE EYE AND ITS APPENDAGES.

The organ of vision consists of the eyeball, the optic nerve, the eyelids, and the lacrymal glands.

## THE EYEBALL.

The eyeball (bulbus oculi) is a hollow globe, which encloses partly formed, partly fluid contents. The walls of the eyeball are composed of three coats: (1) the tunica externa, a fibrous membrane in which an anterior transparent division, the *cornea*, may be distinguished from the remaining opaque portion, the *sclera;* (2) the tunica media, rich in blood-vessels, which includes three divisions, the *choroid*, the *ciliary body*, and the *iris;* (3) the tunica interna, the *retina*, which contains the specialized terminal apparatus of the optic nerve. The formed contents within the eyeball are the *lens* and the *vitreous body.*

## THE TUNICA EXTERNA.

The *cornea* consists of five strata, which enumerated from before backward are the following :—

1. The corneal epithelium.
2. The anterior elastic lamina.
3. The substance proper.
4. The posterior elastic lamina.
5. The corneal "endothelium."

The *corneal epithelium* is a stratified scaly epithelium and consists of a lowermost layer of sharply-contoured columnar cells, which is followed by three or four (more in animals) layers of spherical cells, that in turn are covered by several strata of flattened elements still possessing nuclei. The thickness of the epithelium in man is 0.03 mm. At the rim of the cornea the epithelium is continuous with that of the conjunctival sclera.

The *anterior elastic lamina* (Bowman's membrane, anterior basal membrane) in man is a distinctly-visible stratum, about 0.01 mm. thick, and almost homogeneous in appearance. The surface is provided with minute serrations and ridges for the attachment of the columnar cells of

the corneal epithelium. Posteriorly it gradually passes into the substantia propria of the cornea, of which it is a special modification.

The *substance proper* (substantia propria corneæ) constitutes the chief bulk of the cornea. It consists of delicate parallel fibrillæ, which are united by an interfibrillar cement-substance into bundles of nearly uniform thickness; the bundles in turn are united by an interfascicular cement-substance into flat lamellæ, which lie in many superposed strata and are held together by an interlamellar cement-substance. The lamellæ are arranged parallel to the surface of the cornea and run in

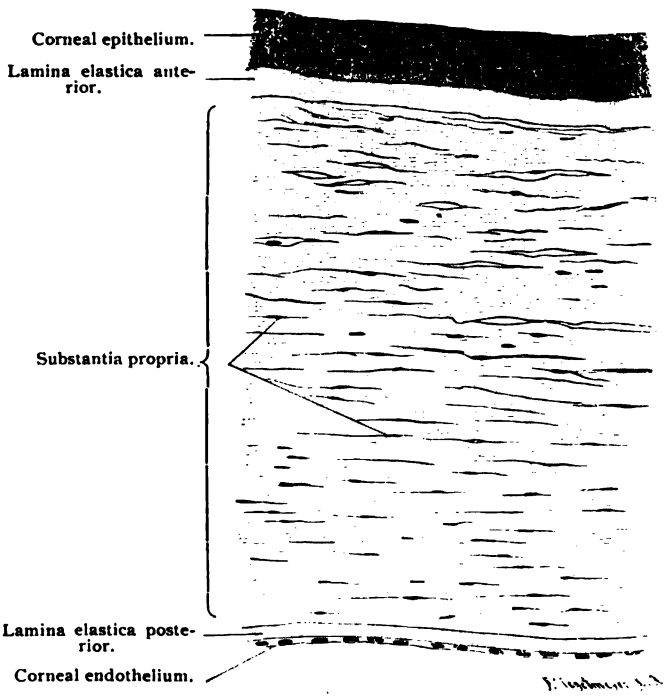


FIG. 253.—VERTICAL SECTION OF A HUMAN CORNEA. × 100. Techn. No. 169 *b*.

meridional curves one above the other, so that a vertical section through the center of the cornea shows alternate longitudinal and transverse bundles. A number of bundles running obliquely, the so-called arcuate fibers, unite each lamella with its neighbor above or below; especially well-developed arcuate fibers occur in the anterior strata of the substantia propria.

Embedded in the cement-substance is an intercommunicating system of much-branched canaliculi, the *corneal canaliculi*, *lymph-canaliculi*, which at many places are expanded to broad oval lacunæ, the *corneal*

*spaces*, *lymph-spaces* (Fig. 254). The latter lie between the lamellæ, while the canaliculi also penetrate between the bundles. The lacunæ and canaliculi contain a serous fluid and cells, "fixed" *corneal corpuscles*

Corneal canaliculi. Corneal spaces.

FIG. 254.—HORIZONTAL SECTION OF THE CORNEA OF AN OX. Silver-preparation; negative picture; the canalicular system is light upon a dark ground. × about 240. Techn. No. 173.

Corneal corpuscles.

FIG. 255.—HORIZONTAL SECTION OF THE CORNEA OF A RABBIT. Positive picture of the corneal canaliculi. × about 240. Techn. No. 174.

and *wandering cells*. The corneal corpuscles are flattened connective-tissue cells possessing large nuclei (Fig. 255); they lie against one wall of the lacunæ.

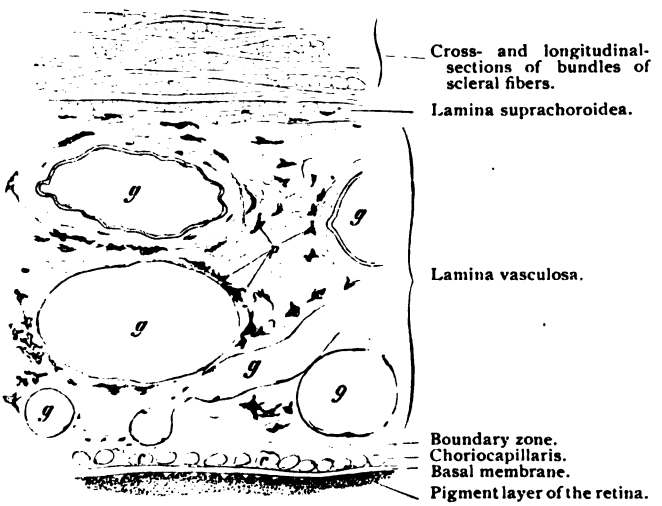


FIG. 256.—VERTICAL SECTION THROUGH A PART OF THE HUMAN SCLERA AND THE ENTIRE CHOROID. × 100. *g*. Larger vessels; *p*, pigment cells; *c*, cross-sections of capillaries. Techn. No. 169 *c*.

The *posterior elastic lamina* (membrane of Descemet, posterior basal membrane) is a transparent elastic layer, only 0.006 mm. thick. In

adult man the posterior surface, at the periphery of the cornea, is beset with hemispherical protuberances.

The *corneal endothelium* is composed of a single layer of flat, polygonal cells, with often slightly-projecting nuclei.

The *sclera* principally consists of interlacing bundles of connective tissue, extending for the most part in meridional and equatorial directions. In addition, delicate elastic fibers arranged in networks and flattened connective-tissue cells are present; the latter, like the corneal corpuscles, lie in lacunæ, that differ from the corneal spaces only in having more irregular outlines. Between the sclera and the choroid is a layer of loose, highly-elastic tissue containing branched pigmented cells and flattened elements free from pigment ("endothelial" cells), which on separating the two coats adheres partly to the former and partly to the latter; the portion on the sclera is called the *lamina fusca scleræ*, that on the choroid, *lamina suprachoroidea*.

The sclera is thickest posteriorly (one millimeter), and becomes gradually thinner toward the cornea.

## THE TUNICA MEDIA.

The *choroid* is characterized by the great abundance of its blood-vessels, which are arranged in two layers. The superficial layer, adjoining the inner side of the lamina suprachoroidea, the *lamina vasculosa* (*layer of*

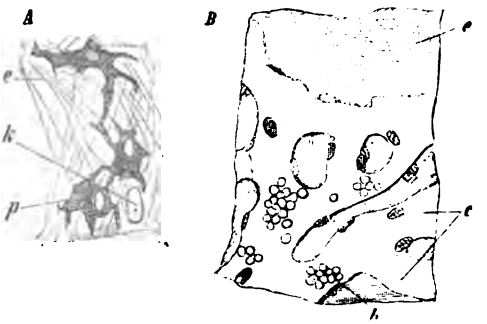


FIG. 257.—*A*. FROM A TEASED PREPARATION OF A HUMAN CHOROID. × 240. *p*. Pigment cells; *e*, elastic fibers; *k*, nucleus of a flat nonpigmented cell; the cell-body is invisible.
*B*. PORTION OF HUMAN CHORIOCAPILLARIS AND THE ADHERENT HYALOID MEMBRANE. × 240. *c*. Wide capillaries, some of which contain (*b*) blood-corpuscles; *e*, hyaline membrane, showing a fine "lattice-work." Techn. No. 170 *a*.

*large blood-vessels*), contains the ramifications of the arterial and venous channels, that are embedded in a supporting tissue, the *stroma*, consisting of networks of fine elastic fibers and numerous branched pigment-cells. In addition, the stroma contains the tissues accompanying the

large arteries; namely, fibrillar connective tissue, smooth muscle-fibers, and nonpigmented plate-like cells that are united in delicate "endothelial" membranes. The deeper layer, the *lamina choriocapillaris*, or layer of capillary networks, is composed of a narrow-meshed net of capillaries, between which no formed elements are found. Between the two laminæ of blood-vessels lies the *boundary zone*, a portion of the stroma consisting of networks of fine elastic fibers and almost devoid of pigment. In ruminants and horses this zone consists of wavy bundles of connective tissue, to which is due the metallic reflex seen in the eyes of these animals. This shining membrane is known as the *tapetum fibrosum*. The similar iridescent *tapetum cellulosum* of carnivora is composed of several strata of plate-like cells containing numerous minute crystals.

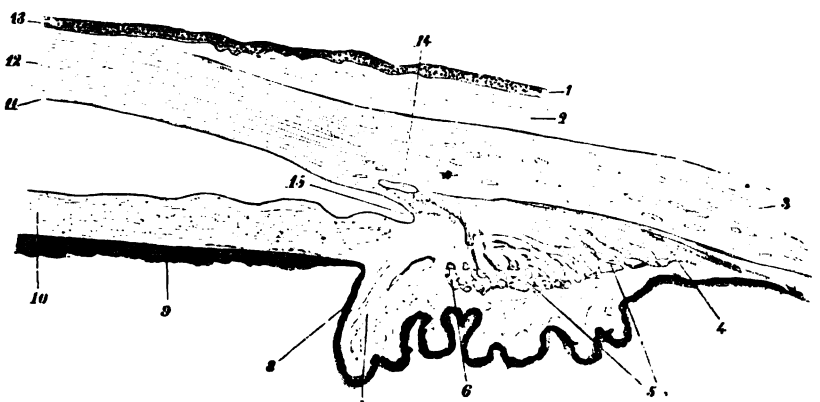


FIG. 258.—MERIDIONAL SECTION THROUGH THE RIGHT IRIDO-CORNEAL ANGLE OF MAN. × 30. 1. Epithelium, 2, connective tissue of the conjunctiva. 3, Sclera. 4, 5, 6, 7, and 8. Ciliary body; 4, meridional, 5, radial, 6, circular fibers of ciliary muscle; 7, ciliary process; 8, ciliary portion of retina. 9. Iridal portion of retina. 10. Stroma of the iris. 11, 12, and 13. Cornea; 11, posterior elastic lamina; 12, substantia propria; 13, epithelium. 14. Venous sinus of sclera. 15. Angle of iris. Techn. No. 169 *a*.

Attached to the lamina choriocapillaris is the *lamina basalis* or vitreous lamina, a structureless lamella about 2 $\mu$ thick, which on its outer surface is provided with delicate lattice-like markings. The polygonal areas noticeable on its inner surface are imprints of retinal pigment. The vitreous membrane approaches in character the elastic membranes.

The *ciliary body* is formed by the ciliary processes and the muscular ring lying upon them, the ciliary muscle. The *ciliary processes* are seventy or eighty meridionally-placed folds, which begin low at the ora serrata, gradually attain a height of one millimeter, and terminate with an abrupt descent near the edge of the lens. Each ciliary process consists of fibrillar connective tissue containing numerous blood-vessels and inwards is limited

by a continuation of the vitreous membrane, that here is distinguished by minute intersecting folds. The blood-vessels of the ciliary processes supply the intraocular fluid. The *ciliary muscle* is an annular band about 3 mm. broad, anteriorly 0.8 mm. thick, arising from the inner wall of the venous sinus of the sclera. The nonstriped elements of which it is composed extend in three different directions. We distinguish (*a*) *meridional fibers* (Fig. 258, 4), numerous fasciculi lying next to the sclera, which reach to the smooth portion of the choroid and are known as the tensor choroideæ; (*b*) *radial fibers*, lying next to the meridional bundles, which from without inward progressively assume a more radial disposition (oriented to the center of the bulbus oculi) and posteriorly, still in the region of the ciliary body, turn and follow a circular course (5); (*c*) *circular (equatorial) fibers*, the so-called *ring-muscle of Müller* (6).

The *iris* consists of a stroma divided in three layers, covered anteriorly

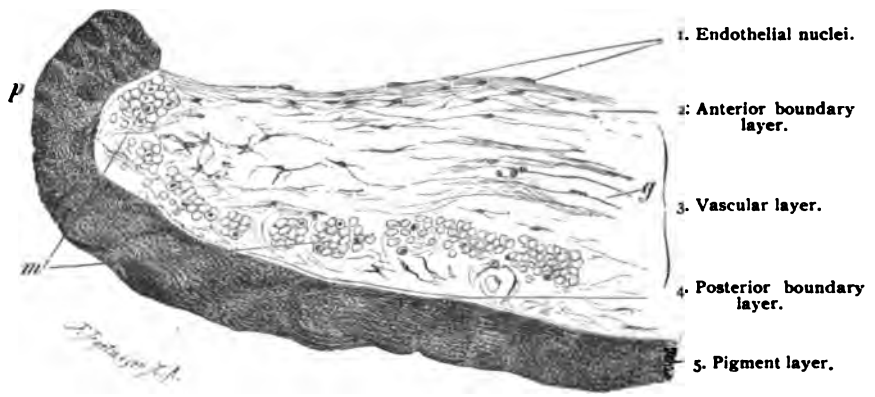


FIG. 259.—VERTICAL SECTION OF THE PUPILLARY PORTION OF A HUMAN IRIS. × 100. About one-fifth of the entire width of the iris is shown. *g*. Blood-vessel, with thick connective-tissue sheath; *m*, sphincter pupillæ muscle cut transversely; *p*, pupillary border of the iris. Techn. No. 170 *c*.

by a continuation of the posterior endothelium of the cornea and posteriorly by a modified extension of the retina. Accordingly five layers may be distinguished:—

1. The anterior "endothelium."
2. The anterior boundary layer.
3. The vascular layer.
4. The posterior boundary layer.
5. The pigment layer.

The *anterior endothelium* covers the anterior surface of the iris and, like that on the posterior surface of the cornea, consists of a single layer of flattened polygonal cells.

The *anterior boundary layer* (reticular layer) comprises three or four

strata of networks, which are formed by stellate connective-tissue cells; it resembles the reticulum of adenoid tissue. The posterior stratum gradually passes into the adjoining vascular stroma.

The *vascular layer of the iris* contains numerous radially-disposed (to the pupil) blood-vessels embedded in a stroma consisting of slender, loosely-united bundles of connective tissue. The blood-vessels and nerves are provided with conspicuously thick connective-tissue sheaths. There are smooth muscle-fibers in the vascular stroma, arranged in two sets, (1) as *annular bundles* encircling the pupillary margin of the iris in a zone about one millimeter in width, constituting the *sphincter of the pupil*, and (2) as a few radially-disposed bundles, which do not form a continuous layer, the *dilator of the pupil*. In the anterior boundary layer and in the vascular stroma pigmented cells occur in greatly varying numbers; in blue eyes they are absent.

The *posterior boundary layer* is a clear, glassy, homogeneous membrane, elastic in its nature.

The *pigment layer of the iris* (pars iridica retinæ) comprises two layers, of which the anterior contains spindle-shaped, the posterior polygonal pigment-cells. Both layers are so crowded with pigment-granules that recognition of the individual elements is almost impossible. The pigment is wanting only in albinos. The posterior surface of the pigment layer is covered by an exceedingly delicate membrane, the *membrana limitans iridis*, a continuation of the membrana limitans interna retinæ.

*The Irido-corneal Angle.*—The junction of the sclera and the cornea is of especial interest, since here the iris, the cornea, and the ciliary body meet. The transition of the sclera into the cornea is absolutely direct; the more wavy bundles of the sclera without interruption in continuity pass over into the straight bundles of the cornea, the system of canaliculi of the sclera communicates with that of the cornea. The line of transition is oblique and microscopically not sharply defined, because the transformation of the sclera into the tissues of the cornea takes place sooner in the posterior than in the anterior strata of the tunica externa. At the periphery of the cornea the posterior elastic lamina and the hindermost laminæ of the substance proper meet the ciliary border of the iris and form the *irido-corneal angle* (Fig. 258, 15). Here the iris sends toward the posterior surface of the posterior elastic lamina connective-tissue processes, *the iridal processes*, that, well developed in animals (cattle, horses), constitute the so-called *ligamentum iridis pectinatum*. In man these processes are inconspicuous. At the periphery of the cornea the posterior elastic lamina splits into fibers which,

strengthened by contributions from the intramuscular connective tissue of the ciliary muscle and from the elastic tendons, also with accessions in a lesser degree from the sclera, blend with the iridal processes. The tissues participating in the formation of the loose mass of fibers occupying the angle of the iris are derived from the structures that meet one another at the irido-corneal angle : cornea, sclera, iris, and ciliary muscle. The posterior endothelium of the cornea continued on to the surface of the iris forms a sheath for these fibers. The spaces between them, that stand in open connection with the anterior chamber of the eye and contain the same fluid, are called the *spaces of Fontana*. In man they are scarcely developed.

## THE TUNICA INTERNA.

The *retina* extends from the entrance of the optic nerve to the pupillary margin of the iris and in this tract three zones may be distinguished : (1) the *pars optica retinæ*, the entire expanse of the optic nerve ;

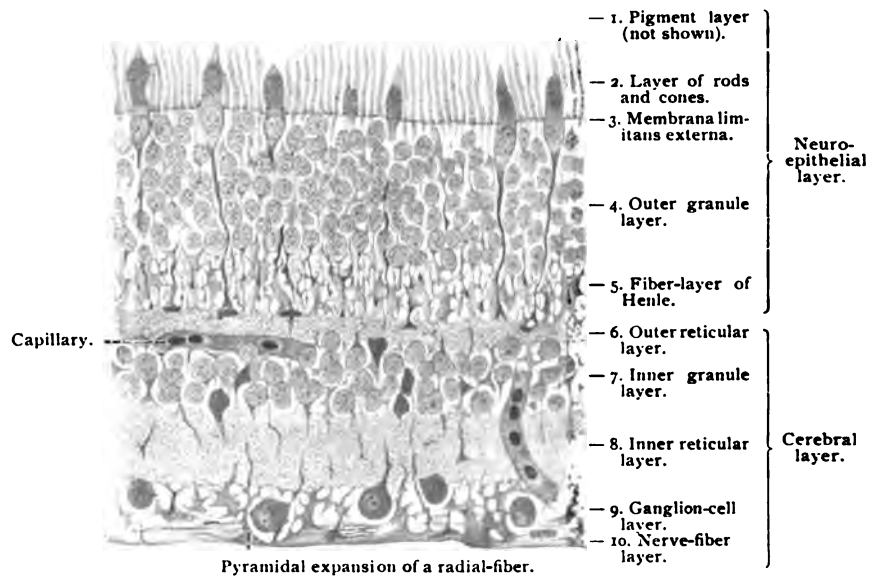


FIG. 260.—VERTICAL SECTION OF A HUMAN RETINA, FROM THE POSTERIOR PORTION OF THE EYEBALL. × 400.—(*After Schaper.*)

(2) the *pars ciliaris retinæ*, extending from the ora serrata to the ciliary margin of the iris ; (3) the *pars iridica retinæ*, which covers the posterior surface of the iris from the ciliary to the pupillary margin.

The *pars optica retinæ*, the portion of the retina alone sensitive to light, lines the entire posterior segment of the eyeball and extends to

within a short distance of the ciliary body, where it terminates in a sharp, macroscopically perceptible, serrated line, the *ora serrata*. It falls into two divisions, an outer *neuro-epithelial lamina*, and inner *cerebral lamina*. In each of these divisions several layers may be distinguished, four in the neuro-epithelial lamina, five in the cerebral lamina; if the pigment layer (pigment-epithelium) lying close beneath the choroid, that genetically belongs to the retina, is added, there are ten layers, that from without inward are arranged in the following order:—

1. The pigment layer.
2. The layer of rods and cones.
3. The membrana limitans externa. } Neuro-epithelial layer.
4. The outer granule layer.
5. The fiber-layer of Henle.
6. The outer reticular layer.
7. The inner granule layer.
8. The inner reticular layer. } Cerebral layer.
9. The ganglion-cell layer.
10. The nerve-fiber layer.*

The elements of the preceding layers are only in part nervous or epithelial in their nature; the other part is formed of supporting substance, that however is not of the nature of connective tissue (p. 169). The most conspicuous elements of the supporting tissue are the *radial fibers* (Müller), elongated cells which extend from the inner surface of the retina through all the layers to the rods and cones. The inner end of the fibers is characterized by a conical foot, the *radial-fiber pyramid;* the expanded bases of these pyramids are so closely placed beside one another that they apparently produce a continuous membrane on the inner surface of the retina, the so-called membrana limitans interna. From the apex of the pyramids the radial fibers, with progressive decrease in thickness, proceed through the inner reticular layer to the inner granule layer, where they are provided with a nucleus; from here they pass

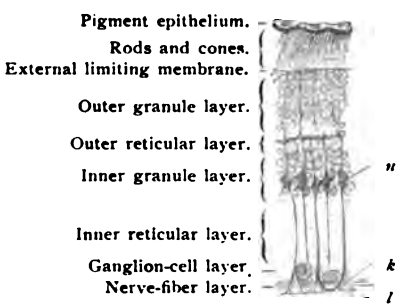


FIG. 261.—VERTICAL SECTION OF THE RETINA OF A RABBIT. × 240. *k*. Expanded base of radial fibers; *n*, nucleated portion of the same; *l*, "membrana limitans interna." Techn. No. 170 *d*.

* To these the membrana limitans interna is sometimes added as an eleventh layer, but it does not represent an independent membrane.

through the outer reticular and outer granule layer to the external limiting membrane, with which they unite. Throughout their entire course the radial fibers give off lateral processes for the support of the nervous elements. In addition to these radial supporting cells, *concentric supporting cells* are found in the outer reticular layer; they extend parallel to the surface, are provided with long processes, are partly nucleated, partly nonnucleated. Glia-cells occur in the vicinity of the optic entrance. From the surface of the membrana limitans externa delicate processes extend to the rods and cones, the bases of which they embrace as the so-called *fiber-crates* (Fig. 262). A portion of both the reticular layers belongs to the supporting substance, as also the small quantity of cement-substance in the ganglion-cell layer.

In the more detailed description of the individual layers of the retina the series will be taken up in the reverse order, from within outward.

### The Cerebral Layer.

The *nerve-fiber layer* consists of naked axis-cylinders which, arranged in bundles, are united in a sort of plexus. From the entrance of the optic nerve, where the fiber-layer is thickest, the fibers expand in a radial direction to the ora serrata. The radial arrangement of the fibers is disturbed in the region of the macula lutea. The majority of the axis-cylinders are centripetal fibers, which originate in the ganglion-cells of the retina; the smaller portion are the axis-cylinder processes of cerebral ganglion-cells, centrifugal fibers, which ramify in the inner granule layer and terminate in free endings.

The *ganglion-cell layer* ("ganglion nervi optici") consists of a single row of large multipolar ganglion-cells,* which send one *unbranched* axis-cylinder process (nerve-process) centralward, toward the nerve-fiber layer, one or more *branched* protoplasmic processes (dendrites) peripheryward, toward the inner reticular layer; there they divide and are arranged in delicate networks lying parallel to the surface, which with the processes from other ganglion-cells form a dense nervous tangle (Fig. 262).

The *inner reticular layer* ("neurospongium") consists of an exceedingly delicate network of supporting tissue, which sustains a dense fiber-maze in the formation of which processes of all the ganglion-cells of the retina participate.

The *inner granule layer* includes elements that differ greatly in their

* A few of these cells are marked by their large size; such giant-ganglion-cells occur at tolerably regular intervals.

nature. The innermost stratum consists of large ganglion-cells,* which send branched processes into the inner reticular layer. From many of these cells—but not all—a nerve-process passes to the optic-fiber layer (Fig. 262). The remaining strata, for the greater part, are composed of small bipolar ganglion-cells (ganglion retinæ), the central process of which extends into the inner reticular layer and there breaks up into delicate varicose branches, while the peripheral process passes to the outer reticular layer; there it divides into forks, spreads out parallel to the surface and resolves into extremely minute fibrillæ which pass into

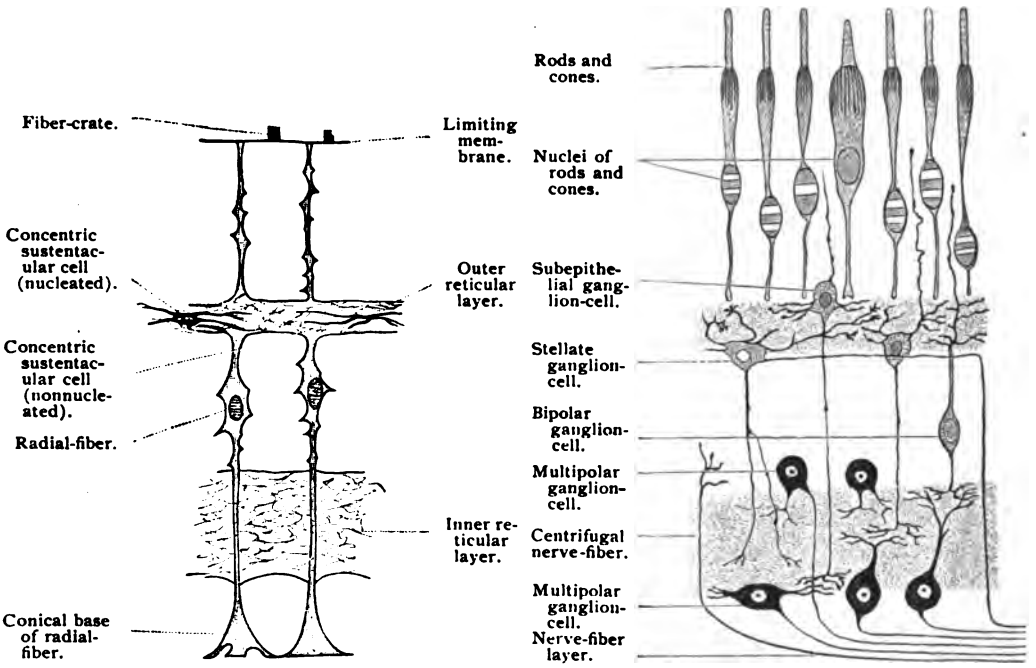


FIG. 262.—SCHEME OF THE ELEMENTS OF THE RETINA; the figure on the left represents the supporting elements, that on the right the nervous and epithelial elements.

a subepithelial tangle formed by the felting of processes of neighboring ganglion-cells. All bipolar ganglion-cells send up one process between the visual cells, that near the membrana limitans terminates in a minute knob (Fig. 262). Finally, the nuclei of the radial fibers occur in this layer.

At the border of this zone, next to the outer reticular layer, lie

* These cells were formerly called spongioblasts, because they were erroneously regarded as the producers of the "neurospongium"; they are elements of the ganglion of the optic nerve which, unlike the other elements, have not wandered through the inner reticular layer.

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small and large stellate cells; they send many processes to participate in the formation of the subepithelial network; one process runs to the inner reticular layer, where it terminates in minute branches, and another —the nerve-process—after a long horizontal course, bends and passes in a vertical direction to the nerve-fiber layer.*

The *outer reticular layer* (subepithelial layer) likewise is a delicate network of sustentacular tissue, which supports the nervous tangle just described. The cellular elements of this layer include the concentric sustentacular cells and the "subepithelial ganglion-cells"; the latter are dislocated elements of the ganglion retinæ, that differ from the bipolar ganglion-cells only in their rounded form, entirely agreeing with the latter in regard to their terminal ramifications (Fig. 262).

### The Neuro-epithelial Layer.

The neuro-epithelial layer consists of two kinds of elements, the *rod-visual cells* and the *cone-visual cells*, that are characterized by the situation of the nucleus in the lower half of the cell and the sharp demarcation of the upper nonnucleated division from the lower portion by the perforated membrana limitans externa. This gives rise to the appearance of different layers, the inner nucleated portion of the visual-cells being known as the outer granule layer, the outer nonnucleated division as the layer of rods and cones. Between these two lies the limiting membrane.

*The Rod-visual Cells.*—The outer halves of these elements are the *rods*, slender cylinders (60 $\mu$ long, 2 $\mu$ thick), which consist of a homogeneous outer segment and a finely-granular inner segment. The outer segment is the exclusive seat of the *visual purple*. The inner segment possesses in its outer end an ellipsoidal, fibrillated body, the *fiber-body*. The inner halves of the rod-visual cells are named *rod-fibers;* they are exceedingly delicate filaments which are provided with nucleated expansions, the *rod-granules*. The nuclei are marked by from one to three clear transverse bands. The basal end of the cell is prolonged as a minute process terminating in a free, club-shaped expansion (Fig. 262).

*The Cone-visual Cells.*—The outer halves of these cells, the *cones*, likewise consist of an outer segment and an inner segment. The outer segments are conical and shorter than those of the rods. The inner segments are thick and expanded; therefore the cone as a whole is flask-shaped. The inner segment of the cones also contains a fiber-body.

* According to other authors this process ends in the outer reticular layer, where its ramifications surround the base of the visual cells.

The inner halves of the cone-visual cells are the *cone-fibers;* these are broad and rest with an expanded pyramidal foot on the outer reticular layer. The nucleated enlargement, the *cone-granule*, usually lies immediately to the inner side of the membrana limitans.

The number of the rods is much greater than that of the cones. The latter occur at regular intervals, so that three or four rods always lie between two cones (Fig. 260).

The basal portions of the visual cells resting upon the outer reticular layer usually are plainly to be recognized as a special radially-striated layer (Fig. 260), *Henle's fiber-layer;* in the region of the macula lutea this fiber-layer is particularly broad and gradually diminishes—often very unsymmetrically—toward the ora serrata.

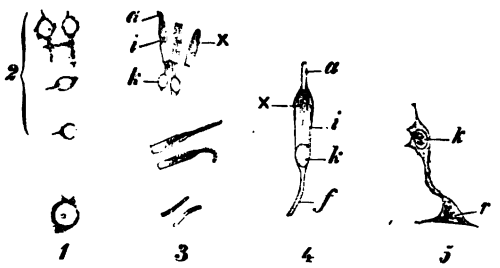


FIG. 263.—ISOLATED ELEMENTS OF THE RETINA OF AN APE. × 240. 1. Mutilated ganglion-cell of the ganglion of the optic nerve. 2. Elements of the inner granule layer. 3. Rod-visual cells and fragments of the same; below, two outer segments, one of which exhibits transverse striation, the beginning of a disintegration into transverse platelets; above are two rods, the outer segment of the lower one falling apart. The uppermost figure shows more complete rod-cells; *a*, outer segment; *i*, inner segment; *k*, nucleus of rod; *x*, fiber-body. 4. Cone-visual cells: *a*, outer segment; *i*, inner segment; *k*, nucleus of cone; *f*, cone-fiber, torn at lower end; *x*, fiber-body. 5. Radial-fiber, *k*, nucleus of the same; *r*, pyramidal base of radial-fiber. Techn. No. 172.

The *pigmented epithelium* consists of a simple layer of hexagonal cells, which on their outer surface, toward the choroid, where the nucleus lies are free from pigment, while their inner division contains numerous rod-shaped pigment-granules, from 1 to 5 $\mu$ long. From the inner division numerous delicate processes extend between the rods and cones. In albinos and on the tapetum the epithelium is free from pigment.

In the region of the macula lutea and fovea centralis, also of the ora serrata, the structure of the retina above described presents modifications calling for special consideration.

*Macula Lutea and Fovea Centralis.*—In the region of the macula the layers of the retina exhibit the following variations. Delicate fibers of the optic nerve run direct from the optic entrance to the adjacent median portion of the macula; above and below these fibers, thicker nerve-fibers run from the optic entrance convexly upward and downward and unite at the lateral margin of the macula. The ganglion-cell layer is greatly

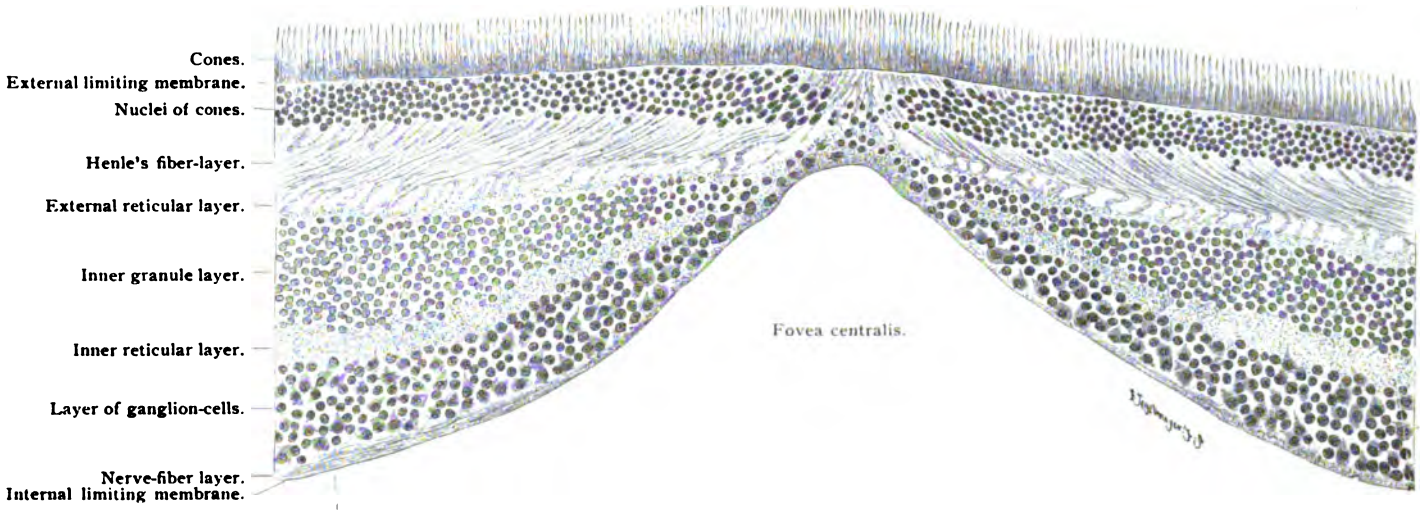


FIG. 264.—HORIZONTAL SECTION THROUGH THE MACULA AND THE CENTER OF THE FOVEA OF A MAN SIXTY YEARS OLD.—(*After Schaper.*) × 135. The nerve-fiber layer, like all the layers, is thicker on the side toward the entrance of the optic nerve than on the opposite side; in the latter situation the nerve-fibers are seen in transverse section as minute dots.

increased in thickness, owing to the development of the layer of bipolar ganglion-cells, which instead of a single row are arranged in many (up to nine) rows; also the inner granule layer by multiplication of its elements is almost twice as broad. The inner and outer reticular layers suffer no essential change. The neuro-epithelial layer is here represented by the somewhat smaller cone-visual cells alone. Already at the margin of the macula the rod-visual cells diminish in number and within the macula they are wanting altogether; as a result the cone-fibers are visible in a wide extent; here they alone form the fiber-layer of Henle. The cone-granules, on account of their large number, lie in several rows one above the other. The radial-fibers no longer stand vertically to the thickness of the retina, but obliquely toward the fovea.

Toward the *fovea centralis* situated in the center of the macula the layers of the retina become gradually thinner and are in part totally suspended. With the exception of a few fibers, the nerve-fiber layer first disappears; then the cerebral layers fuse with one another and in the center of the fovea with the cone-granules, forming a thin layer in which the boundaries of the individual strata can no longer be recognized. In the center of the fovea (fundus foveæ) the neuro-epithelial layer (cone-cells) almost alone is present.

A diffuse yellow pigment permeates the cerebral layer, but is absent in the neuro-epithelial layer; therefore the fundus foveæ is colorless.

In the region of the *ora serrata* a rapid diminution in the retinal layers takes place. Optic-fibers and ganglion-cells disappear before reaching the ora serrata. Of the visual cells the rod-visual cells are the first to vanish; the cone-visual cells are still preserved, but appear to be deprived of their outer segment. Then the outer reticular layer is lost, so that the outer and inner granular layers become confluent, and finally the inner reticular layer ceases. The radial fibers of Müller, on the contrary, persist and are highly developed. [Within the region of the ora serrata commonly smaller or larger clefts or even rather voluminous spaces occur, which are called *vacuoles* (Fig. 265). They are either confined to the neuro-epithelial layer or may extend centrally into the inner reticular layer. They are probably filled with a lymphatic fluid. The meaning of these spaces is unknown, but they are certainly not to be regarded as pathological or senile changes, because they are rather common in the perfectly-normal retinæ of young individuals.—EDITOR.]

The *pars ciliaris retinæ* consists of a simple layer of slender columnar cells, which gradually originate in the blended inner and outer granule layers (Fig. 265). These cells are covered on their centrally-directed surface by a cuticular membrane, a true membrana limitans

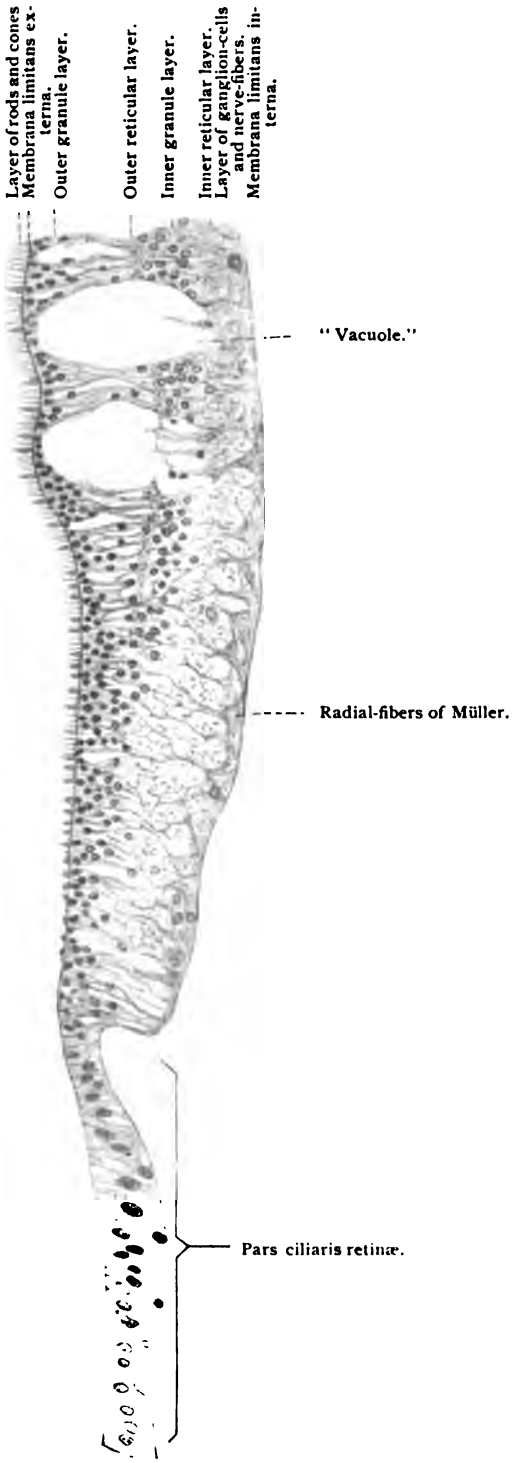


FIG. 265.—MERIDIONAL SECTION OF THE ORA SERRATA AND THE ADJACENT PORTION OF THE PARS CILIARIS RETINÆ OF A MAN THIRTY-SEVEN YEARS OF AGE. × 180.—(*Schaper.*)

interna, which is not present in the pars optica retinæ ; their peripheral surface is joined to pigmented cells, a continuation of the pigmented epithelium.

The *pars iridica retinæ*, the pigment layer of the iris, has been described (p. 347).

With regard to the *connections of the nervous elements of the retina*, according to the foregoing description the nerve-processes of the ganglion-cells of the ganglion of the optic nerve and of the stellate cells of the inner granule layer are the centripetal optic-fibers, while the centrifugal nerve-fibers terminate in free endings in the inner granule layer. The ganglion-cells of the ganglion retinæ apparently do not possess a nerve-process ; their union with the other nervous elements is effected by means of the nervous tangles in the two reticular layers, and not only as elsewhere by contact in the customary manner (p. 102), but also by direct connection by means of true anastomoses.*

The connection with the visual cells is effected by means of the intra-epithelial processes of the cells of the ganglion retinæ, that terminate between (not within) the visual elements. Physiologic researches make it highly probable that the visual-cells constitute the essential percipient part of the retina.

## THE OPTIC NERVE.

The *optic nerve* in its entire intraorbital course is enveloped in sheaths which are processes of the cerebral membranes. Outermost is the compact dural sheath, consisting of longitudinally-disposed bundles of connective tissue (Fig. 266) ; following within this is the exceedingly delicate arachnoidal sheath, which sends numerous relatively thick connective-tissue trabeculæ inward to the pial sheath, while the union with the dural sheath is represented by a few delicate fibers. Innermost lies the pial sheath, which closely invests the optic nerve and sends numerous septa between the individual nerve-fiber bundles. These septa are connected with one another by transverse trabeculæ, the resultant structure being a transverse lattice-work.

The tissue of the pial sheath does not penetrate within the nerve-fiber bundles, but only forms an outer envelope for them. The nerve-fiber bundles consist of medullated fibers without a neurilemma ; they are held together by many neuroglia-cells (spider cells). At the entrance of the optic-nerve into the eyeball the dural sheath passes into the sclera,

* Not shown in Fig. 262.

the arachnoidal sheath, at its anterior border, resolves into fibers, so that the subdural space lying on its outer side communicates with the subarachnoidal space on its inner side. The pial sheath blends with the sclera, which here is pierced with numerous apertures for the nerve-fibers passing through it ; this portion of these sheaths is called *lamina cribrosa*. The choroid also participates, though in a slight degree, in the formation of the lamina cribrosa. The nerve-fibers lose their medullary sheath

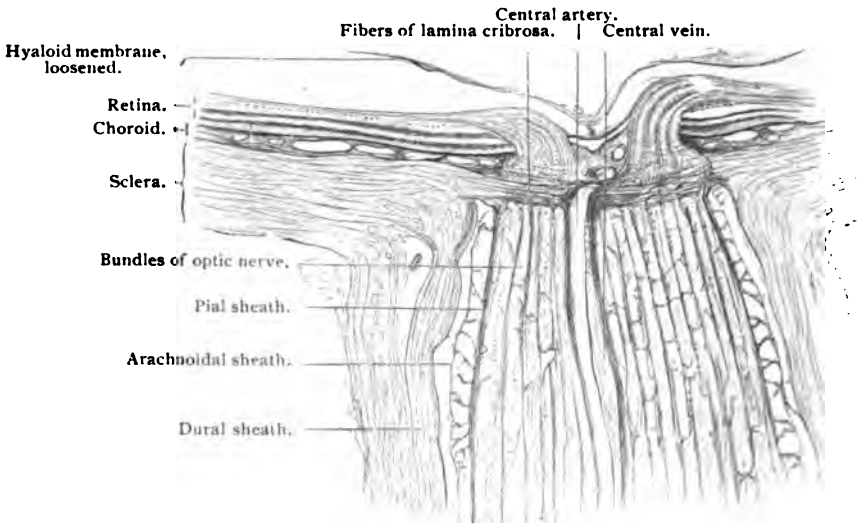


FIG. 266.—LONGITUDINAL SECTION OF THE OPTIC ENTRANCE OF A HUMAN EYE. × 15. Above the lamina cribrosa the narrowing of the optic nerve is visible. The central artery and vein have been for the most part cut longitudinally, but above at several points transversely. Techn. No. 169 *d*.

at the point of entrance and consequently the nerve is considerably reduced in size.

The central artery and vein of the retina lie in the axis of the distal half of the optic nerve; the connective tissue investing these vessels is connected at many points with the pial sheath, as well as with the lamina cribrosa.

## THE LENS.

The lens consists of a substantia lentis that on its anterior surface is covered by the epithelium of the lens ; the whole is enveloped by the lens-capsule. In the *substantia lentis* a soft cortical substance and a firm core may be distinguished ; it consists throughout of colossal, greatly-elongated epithelial-cells, the *lens-fibers*. They have the form of six-sided prismatic bands, that are thickened at their posterior extremities. The lens-fibers of the cortical substance have smooth borders and in the

vicinity of the equator lies an oval nucleus. The lens-fibers of the central portion of the lens have dentated borders and are nonnucleated. All the fibers are united with one another by a small amount of cement-substance, that is accumulated in larger quantities at the anterior and posterior poles of the lens and gives rise to the so-called anterior and posterior *lens-stars*, stellate forms seen in macerated preparations. All the lens-fibers, beginning at the anterior lens-star, run in a meridional direction to the posterior lens-star; but no lens-fiber spans the entire half of the lens; the nearer the fibers arise to the anterior pole, the more remote from the posterior pole do they find their termination.

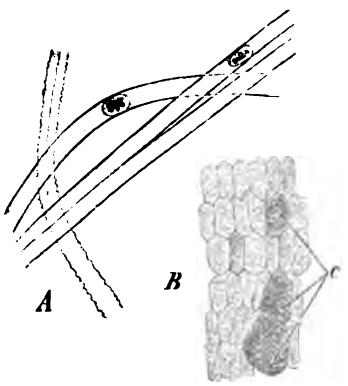


FIG. 267.—LENS-FIBERS OF AN INFANT. *A*. Isolated lens-fibers, three with smooth, one with dentated borders. × 240. Techn. No. 178. *B*. Human lens-fibers cut transversely; *c*, section through club-shaped ends. × 560 Techn. No. 179.

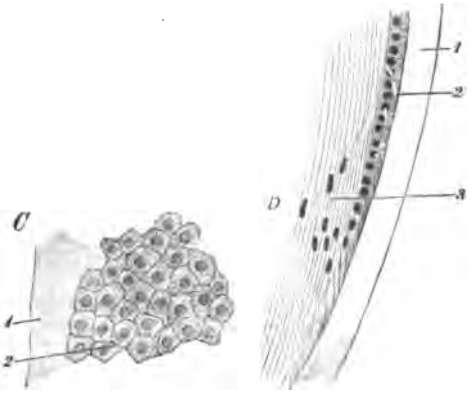


FIG. 268.—CAPSULE AND EPITHELIUM OF AN ADULT HUMAN LENS. *C*. Inner aspect. × 240. Techn. No. 180 *a*. *D*. Lateral aspect, from a meridional section through the equator of the lens; *1*, capsule; *2*, epithelium; *3*, lens-fibers. × 240. Techn. No. 180 *b*.

The *lens-epithelium* consists of a simple layer of cubical cells, which covers the anterior surface of the lens and extends as far as the equator; here the epithelium, with gradual elongation of its elements, is transformed into the lens-fibers (Fig. 268, *D*).

The *lens-capsule* is a transparent, elastic membrane; the *anterior capsule*, the portion covering the anterior surface of the lens, is from 11 to 15 $\mu$ thick, the corresponding posterior portion, the *posterior capsule*, only 5 to 7 $\mu$. The lens-capsule comprises two genetically distinct parts; the one is a cuticular formation, a product of the epithelium of the lens, the other, partly of the nature of connective tissue, is a transformation product of the embryonal connective-tissue sheaths.

## THE VITREOUS BODY.

The *vitreous body* consists of a fluid substance, the *vitreous substance*, and of *fibers*, which extend in all directions through the former. The surface of the vitreous body is covered by a firmer membrane, the *hyaloid membrane*, and in certain localities contains a limited number of fibrillæ and a few cells; of the latter two forms may be distinguished, (1) round elements, resembling leucocytes, and (2) stellate and fusiform cells. Cells containing clear vacuoles probably are degenerating forms.

## THE SUSPENSORY LIGAMENT.

The *suspensory ligament* (zonula ciliaris, zone of Zinn), consists of delicate homogeneous fibers which extend from the surface of the hyaloid membrane, in the vicinity of the ora serrata, in a meridional direction toward the lens. They are attached to the inner surface of the ciliary processes and proceed from the tips of the same over to the equator of the lens, where they are attached to the anterior and posterior surfaces and to the equator of the lens-capsule. The fibers do not anywhere form a continuous membrane, but are radially-plicated extensions of the hyaloid membrane that find attachment on and afford support to the lens. The annular cleft between the zonula ciliaris behind and the vitreous body in front is designated *canal of Petit* (spatia zonularia). Other authors describe the triangular space included between the anterior and posterior zonula fibers and the lens-capsule as the canal of Petit. The canal is not completely closed on the side toward the posterior chamber of the eye.

## THE BLOOD-VESSELS OF THE EYEBALL.

The blood-vessels of the eyeball are separated in two sharply-defined regions, which are in communication only at the entrance of the optic nerve.

I. *Territory of the Vasa Centralia Retinæ* (Fig. 269).—The *central artery of the retina*, at a distance of from 15 to 20 millimeters from the eyeball, enters the axis of the optic nerve (*a*) and runs within it to the surface of the optic entrance. Here it divides into two main branches, of which the one is directed upward, the other downward, and each of which subdividing supplies the entire pars optica retinæ to the ora serrata. During its course in the optic nerve the artery gives off numerous small branches, which run within the processes of the pial sheath between the nerve-fiber bundles and anastomose with small arteries (*b*)

that have entered the sheaths of the nerve from the surrounding adipose tissue and also with twigs of the short ciliary arteries (at *c*). In the retina

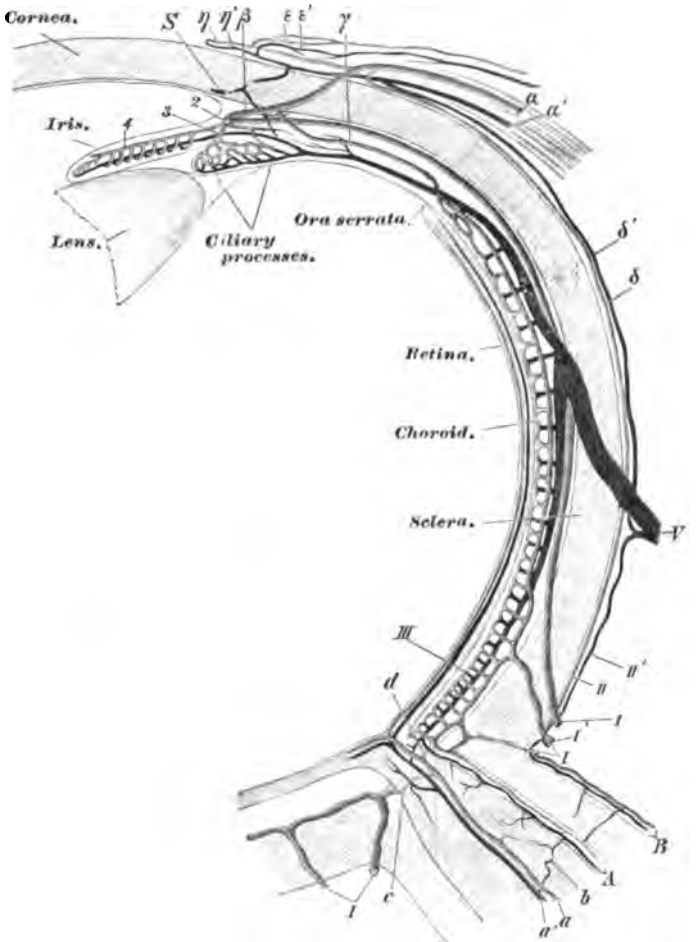


FIG. 269.—SCHEME OF THE VESSELS OF THE EYE, ACCORDING TO LEBER. External tunic stippled, middle tunic white, internal tunic and optic nerve dotted crosswise. Arteries light. Veins dark. Region of the central vessels of the retina (small Italic letters): *a*. Artery; *a'*, central vein of retina; *b*, anastomosis with vessels of the sheath; *c*, anastomosis with branches of the posterior short ciliary arteries; *d*, anastomosis with choroidal vessels. Region of the vessels of the sheath (large Italic letters): *A*. Inner; *B*, outer vessels of the sheath. Region of the posterior short ciliary vessels (Italic numerals): *I*. Arteries; *I'*, veins (short posterior ciliary); *II*, episcleral arterial; *II'*, episcleral venous branches of the same; *III*, capillaries of the choriocapillaris. Region of the posterior long ciliary vessels (Arabic numerals): 1. Posterior long ciliary artery; 2, circulus iridis major cut transversely; 3, branches to the ciliary body; 4, branches to the iris. Region of the anterior ciliary vessels (Greek letters): α, Artery; α', vein (anterior ciliary); β, connection with the circulus iridis major; γ, connection with the choriocapillaris; δ, arterial; δ', venous episcleral branches; ε, arterial; ε', venous branches to the scleral conjunctiva; η, arterial; η', venous branches to the corneal limbus; *V*, vena vorticosa; *S*, cross-section of the venous sinus of the sclera.

itself the artery breaks up into capillaries, which extend into the outer reticular layer. The cerebral layer of the retina alone contains blood-vessels; in the fundus foveæ the cerebral layer is wanting and with it

the blood-vessels. The veins proceeding from the capillaries run parallel with the branches of the arteries and finally unite in the *vena centralis retinæ* enclosed within the axis of the optic nerve (Fig. 269, *a'*).

In the embryo a twig from the central artery of the retina, the *hyaloid artery*, passes through the vitreous body to the posterior surface of the lens. This artery atrophies before birth, but the canal which transmits it may still be found in the vitreous body of the adult; it is called the *hyaloid canal.*

II. *Territory of the Vasa Ciliaria.*—This region is characterized by the complementary veins taking a course entirely different from that of the arteries.

Of the *arteries*, the short ciliary arteries (Fig. 269, Roman numerals) supply the smooth portion of the choroid, while the long ciliary arteries (Fig. 269, Arabic numerals) and the anterior ciliary arteries (Fig. 269, Greek letters) are primarily destined for the ciliary body and iris.

The branches, about twenty, of the *short ciliary arteries* (arteriæ ciliares posticæ breves) penetrate the sclera in the vicinity of the optic entrance (I); after giving off twigs (II) which supply the posterior half of the surface of the sclera, the arteries break up into a narrow-meshed capillary network, the *choriocapillaris* (III). At the optic entrance the arteries anastomose with branches of the arteria centralis retinæ (Fig. 259, *c*) and in this way form the *circular artery of the optic nerve;* at the ora serrata they anastomose with recurrent twigs of the long ciliary and of the anterior ciliary arteries (for the latter anastomosis see Fig. 269, *γ*).

The two *long ciliary arteries* (arteriæ ciliares anticæ longæ) (1) likewise penetrate the sclera at the optic entrance; the one artery passes to the nasal, the other to the temporal side of the eyeball, between the choroid and the sclera to the ciliary body, where each artery divides in two diverging branches running along the ciliary margin of the iris; by the anastomoses of these branches of the two long ciliary arteries a vascular ring (2) is formed, the *larger arterial circle* of the iris (circulus iridis major) from which numerous twigs arise for the ciliary body and ciliary processes (3) and for the iris (4). Near the pupillary margin of the iris the arteries form an incomplete ring, the *smaller arterial circle* (circulus iridis minor).

The *anterior ciliary arteries* (arteriæ ciliares anticæ) come from the arteries supplying the recti muscles of the eye, penetrate the sclera near the corneal margin, communicate with the larger arterial circle of the iris (*β*) supply the ciliary muscle, and send recurrent branches to unite with the choriocapillaris (*γ*). Before the anterior ciliary arteries penetrate

the sclera, they give off twigs toward the *back* for the anterior half of the sclera ($\delta$), toward the *front* to the conjunctival sclera ($\varsigma$) and to the corneal limbus ($\eta$). The cornea itself is without blood-vessels; only at the margin, in the anterior lamellæ of the substantia propria, is there a circumferential network of capillary loops.

All the *veins* run toward the equator, where they converge to four (more rarely five or six) small stems, the whorl veins or *venæ vorticosæ*, which forthwith pierce the sclera (Fig. 269) and empty into one of the ophthalmic veins. In addition to these there are small complemental veins that run parallel to the short ciliary arteries and to the anterior ciliary arteries, the short ciliary veins (Fig. 269, $I'$), and the anterior ciliary veins ($a'$); the anterior ciliary veins receive twigs from the ciliary muscle, from the episcleral vascular network (Fig. 269, $\delta'$), from the conjunctival sclera ($\varepsilon'$), and from the circumferential capillary loops of the cornea ($\eta'$). The episcleral veins also communicate with the venæ vorticosæ at the equator (at $V$). The anterior ciliary veins finally communicate with the *sinus venosus scleræ* (Schlemm) ($S$). This is a *venous wreath* encircling the cornea, that, lying within the sclera, still possesses completely-closed walls.* It takes up small veins from the capillary network of the ciliary muscle.

## THE LYMPH-CHANNELS OF THE EYEBALL.

The eye possesses no proper lymph-vessels, but a series of intercommunicating lymph-spaces. Two complexes of such spaces may be distinguished, an anterior and a posterior tract. The anterior tract comprises:—

1. The *lymph-canaliculi* of the *cornea* and *sclera*.

2. The *anterior chamber* of the eye, which, by means of the capillary cleft between the iris and the lens, communicates with—

3. The *posterior chamber* of the eye. The latter is in open connection with—

4. The *spatia zonularia*.

The last three spaces stand in close relation to one another and may be injected from the anterior chamber.

The posterior tract includes:—

1. The *hyaloid canal* (canalis hyaloideus).

2. The *lymph-clefts* between the sheaths of the optic nerve, the sub-

* The communication with the anterior chamber of the eye formerly described is factitious; the assertion that such communication existed was based on the fact that colored fluids injected into the anterior chamber pass over into the venous-wreath by filtration.

dural and the subarachnoidal spaces, the narrow cleft between the choroid and the sclera, the perichoroidal space, and the spatium interfasciale (Tenon), which extends from the dural sheath of the optic nerve to the optic foramen. These spaces may be filled from the subarachnoidal space of the brain. The content of these spaces is a filtrate from the blood-vessels, which also permeates the vitreous body. The quantity of this fluid in the perichoroidal space, also in the interfascial space, normally is exceedingly scanty. Both these spaces serve to facilitate the movements of the choroid and of the eyeball and may be regarded as synovial spaces.

## THE NERVES OF THE EYEBALL.

The nerves of the eyeball penetrate the sclera in the circumference of the entrance of the optic nerve and run forward between the outer tunic and the choroid; after giving to the choroid bundles provided with ganglion-cells, they form an annular plexus intermingled with ganglion-cells lying upon the ciliary body, the *ciliary ganglionic plexus* (plexus gangliosus ciliaris), from which branches arise for the ciliary body, the iris, and the cornea. The *nerves of the ciliary body* terminate in delicate, pointed ends in the blood-vessels and in the ciliary muscle, partly between the muscle-bundles in the form of branched terminal

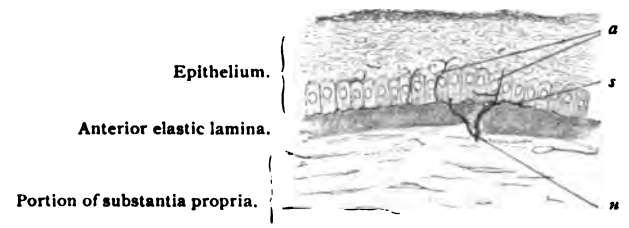


FIG. 270.—FROM A VERTICAL SECTION THROUGH THE HUMAN CORNEA. × 240. *n*. A dividing nerve penetrating the anterior basal membrane; *s*, subepithelial plexus *beneath* the cylindrical cells; *a*, fibers of the intra-epithelial plexus ascending *between* the epithelial-cells. Techn. No. 177.

twigs, which perhaps subserve the muscular sense, partly on the scleral surface of the ciliary body in the form of a delicate plexus. The medullated *nerves of the iris* form networks and lose their medullary sheath as they pass to the pupillary margin; their terminal ramifications are in part distributed to the smooth muscle-fibers and to the blood-vessel walls, while another portion forms a dense sensory plexus lying close beneath the anterior iridal surface. The *nerves of the cornea* first enter the sclera and form a circular plexus, the *plexus annularis*, surrounding the corneal margin, from which branches arise for the sclera and for the cornea. In man the twigs in the sclera terminate in spherical end-bulbs lying close under the

epithelium ; they are also found in the substance proper of the cornea for a distance of from one to two millimeters within the corneal limbus. The branches that go to the cornea, after their entrance in the substance proper, lose their medullary sheath and as naked axis-cylinders penetrate the entire structure. They form networks, which according to the plane they occupy are described as the *stroma-* or *ground-plexus*, which lies in the deeper strata of the cornea ; the *sub-basal plexus*, which is situated beneath the anterior elastic lamina ; the *sub-epithelial plexus*, which lies close under the epithelium. From the latter plexus exquisitely-delicate nerve-fibrillæ ascend into the epithelium between its elements and form the exceedingly fine *intra-epithelial plexus*, the ramifications of which terminate in free ends between the epithelial-cells (Fig. 270).

## THE EYELIDS.

The eyelids, *palpebræ*, are folds of the integument, which enclose muscles, loose and compact connective tissue, and glands. The outer fold of the eyelid retains the usual character of the skin ; the inner fold, that toward the eyeball, is considerably modified and is called the *palpebral conjunctiva*. The skin on the external surface of the eyelid extends over the anterior free margin of the lid and does not pass into the palpebral conjunctiva until it reaches the posterior border, the *palpebral border*.

The eyelid is best studied in a sagittal section (Fig. 271) in which, counting from before backward, the following strata are found :

1. The *integument* is thin and beset with fine lanugo-hairs, the follicles of which it encloses ; in the corium small coil-glands are found, also pigmented connective-tissue cells, that are of rare occurrence elsewhere in the corium. The subcutaneous tissue is very loose, rich in fine elastic fibers, poor in fat-cells, that may be entirely wanting. Near the border of the lid the corium is more compact and beset with more conspicuous papillæ. In the anterior edge of the margin of the lid two or three rows of robust hairs, the *cilia*, are obliquely implanted, the follicles of which extend far into the corium. The cilia undergo rapid shedding ; their length of life is said to be about from one hundred to one hundred and fifty days ; consequently hairs in all stages of development are frequently found among the eyelashes. The hair-follicles of the cilia are provided with small sebaceous glands, in addition to which they take up the excretory ducts of the *ciliary glands* (Moll), which in their minute structure resemble coil-glands, from which they differ only in having their lower ends less convoluted.

2. Posterior to the subcutaneous tissue lie the transverse bundles of cross-striated muscle-fibers of the *orbicularis palpebrarum muscle;* the portion of the muscle lying behind the cilia (McR) is named the tarsal muscle (Riolan).

3. Behind the muscle the fibrous extensions of the tendon of the levator palpebræ muscle are met, which are partly lost in the areolar tissue present, the so-called fascia palpebralis, and partly attached to the

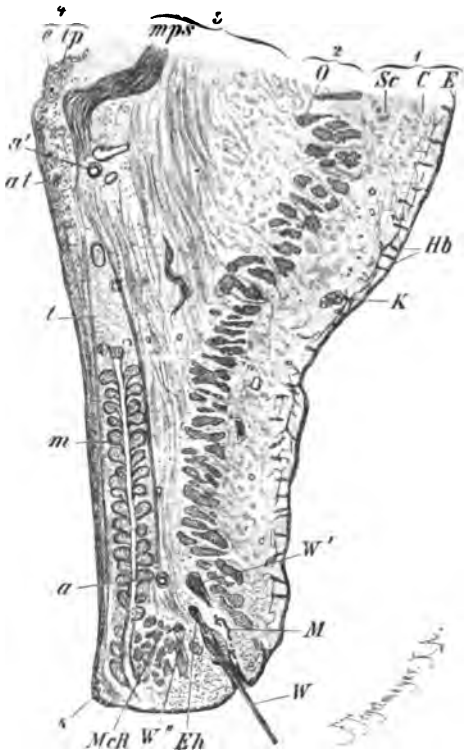


FIG. 271.—SAGITTAL SECTION OF THE UPPER EYELID OF A SIX-MONTHS'-OLD CHILD. × 10. 1. Integument: *E*, epidermis; *C*, corium; *Sc*, subcutaneous tissue; *Hb*, hair-follicles of lanugo hair; *K*, coil-gland; *W*, eyelash, with the anlage of a new hair (*Eh*); *W'*, *W''*, portions of follicles of eyelashes; *M*, portion of a ciliary gland. 2. Region of the orbicularis palpebrarum muscle: *O*, bundles of this muscle cut transversely; *McR*, tarsal muscle. 3. Expanded tendon of the levator palpebrarum superior: *mps*, superior palpebrarum muscle. 4. Conjunctival portion: *e*, conjunctival epithelium; *tp*, tunica propria; *al*, accessory tear-glands; *t*, tarsus; *m*, tarsal glands, the mouth of excretory duct is not shown; *a*, transverse section of the arcus tarseus; *a'*, transverse section of the arcus tarseus externus. 5. Margin of eyelid. Techn. No. 182.

upper margin of the tarsus; the latter portion contains smooth muscle-fibers (*mps*) the *superior palpebral muscle* (Müller). In the lower eyelid the expansion of the tendon of the inferior rectus muscle also contains bundles of nonstriped muscle-fibers, the *inferior palpebral muscle.*

4. The *tarsus* is a plate of dense fibrous tissue, which gives firmness and support to the eyelid. It lies immediately in front of the conjunctiva,

to which it belongs, and occupies the lower two-thirds of the height of the entire eyelid. In its substance the *tarsal glands* (Meibom) (*m*) are embedded, elongated bodies which consist of a wide excretory duct opening on the palpebral border and of little follicles with short stalks, that empty into it on all sides. In their histology the tarsal glands agree with the sebaceous glands. At the upper end of the tarsus, partly enclosed by its substance, lie branched tubular glands which in their minute structure coincide with the tear-glands and therefore are called *accessory tear-glands* (Fig. 261, *at*); they principally occur in the inner (nasal) half of the eyelid.

Behind the tarsus lies the *conjunctiva* proper, which consists of an epithelium (*e*) and a tunica propria (*tp*). The former is a stratified columnar epithelium, with several rows of spherical cells in the depths and a row of mainly short cylindrical cells on the surface. The latter possess a narrow hyaline cuticular border. Goblet-cells also occur in varying numbers. At the posterior palpebral border the epithelium gradually passes into the stratified scaly variety, that occasionally extends far over on the conjunctiva. The lower portion of the palpebral conjunctiva is smooth. In the upper portion, on the contrary, the epithelium forms irregular pocket-like depressions, the "conjunctival recesses," that differ greatly in individual development and in sections, when highly developed, may resemble glands. The tunica propria of the conjunctiva consists of connective tissue, of plasma-cells in varying number, and of lymphoid cells, the number of which likewise varies greatly. In animals, especially in ruminants, the latter form true nodules, the so-called *trachoma* glands, from the summit of which the leucocytes wander through the epithelium to the surface; in man, the migration of the leucocytes occurs in a slighter degree. In the region of the conjunctival recesses, the tunica propria is divided into papillæ by the depressions of the epithelium, hence the name "papillary body."

The palpebral conjunctiva passes from the eyelids to the eyeball, the anterior surface of which it covers. At the point of transit, the *fornix conjunctivæ*, a loose sub-conjunctival tissue consisting of connective-tissue bundles occurs under the tunica propria. The epithelium is the same as that on the palpebral conjunctiva; the tunica propria contains fewer leucocytes, but also in man normally possesses about twenty small lymph-nodules and a few mucous glands. The scleral conjunctiva is modified in so far that the stratified columnar epithelium within a certain distance of the cornea is transformed into the stratified scaly variety, which continues in that of the cornea.

The rudimentary *third eyelid* (plica semilunaris) consists of connective

tissue and stratified squamous epithelium. The *caruncula lacrimalis* resembles the skin in structure, only the stratum corneum is absent, and contains fine hairs, sebaceous and accessory tear-glands.

The *blood-vessels* of the eyelids proceed from branches that, approaching from the outer and inner angles of the eye, form an arch, the *arcus tarseus* (Fig. 271, *a*), at the margin of the lid and a second arch, the *arcus tarseus externus* (*a'*), at the upper end of the tarsus. Branches from these arches ramify in the skin, surround the tarsal glands, and penetrate the tarsus to supply a capillary network lying beneath the conjunctival epithelium; they also supply the fornix conjunctivæ, the scleral conjunctiva, and anastomose with the anterior ciliary arteries.

The *lymph-vessels* form a close-meshed network in the tarsal conjunctiva, a very open-meshed network on the anterior surface of the tarsus. According to some authors, the lymph-channels of the scleral conjunctiva are closed at the corneal limbus; according to others, they send minute canaliculi into the tissue of the cornea and are in communication with the system of lymph-spaces and canaliculi in the latter.

The *nerves* form a very dense plexus in the tarsus and in the palpebral conjunctiva, which is characterized by a peculiar, coil-like, twisted arrangement of its fibers. One portion of the tarsal plexus surrounds the tarsal glands * and here consists of many nonmedullated and few medullated nerve-fibers; another portion terminates in the walls of the blood-vessels. From the "conjunctival" plexus medullated nerve-fibers arise, that run obliquely toward the margin of the lid and the palpebral conjunctiva, lose their medullary sheath, in part penetrate directly into the epithelium, where they branch and terminate in free endings, in part terminate in end-bulbs lying close under the epithelium. These end-bulbs are found in large numbers not only in the papillæ of the margin of the lid and in the palpebral conjunctiva, but also in the ocular conjunctiva and in the margin of the cornea (see also p. 193).

## THE LACRYMAL GLANDS.

The *lacrymal glands* are compound tubular glands, provided with several excretory ducts. The latter are clothed with a two-layered cylindrical epithelium and pass into long, narrow intercalated tubules clothed with low epithelial-cells. These pass into the gland-tubules, which are lined by serous gland-cells.

* Whether nerve-fibers penetrate between the gland-cells has not yet been distinguished with certainty.

The walls of the *lacrymal canaliculi* consist of stratified scaly epithelium, of a tunica propria rich in elastic fibers, beneath the epithelium also rich in cellular elements, and of cross-striped muscle-fibers, for the greater part running longitudinally.

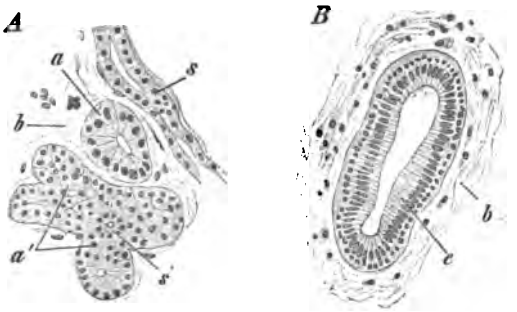


FIG. 272.—FROM A THIN SECTION OF A HUMAN LACRYMAL GLAND. × 240. *A*. Gland; *a*, tubule cut transversely; *a'*, group of tubules, mostly cut obliquely, the lumen of one tubule only visible below; *s*, intercalated tubule with cubical (above to the right) and flat (below to the left), epithelial-cells; *s'*, intercalated tubule in cross-section, lined with moderately high cylindrical cells; *b*, connective tissue. *B*. Cross-section of the duct; *e*, double layer of cylindrical epithelium; *b*, connective tissue. Techn. No. 183.

The lacrymal sac and the naso-lacrymal duct consist of a two-layered columnar epithelium and of a tunica propria which is chiefly adenoid in character and separated from the underlying periosteum by a dense plexus of veins.

## TECHNIC.

No. 169.—Carefully cut the fresh *eye-ball* out of the optic cavity and secure as much as possible of the optic nerve; then with the scissors remove the attached fat and muscle and with a *sharp* razor make an incision at the equator, about 1 cm. long, through all the coats of the eye. Place the eye-ball in 150 c.c. of 0.05 per cent. chromic acid solution (p. 31); after from twelve to twenty hours, beginning at the incision already made, divide the eye-ball with the scissors completely into an anterior and posterior half and change the fluid. After another twelve or twenty hours wash the pieces and harden them in 100 c.c. of gradually-strengthened alcohol (p. 33).

*a*. Carefully remove the lens from the anterior half of the eye-ball and treat it further like No. 181; then cut out a quadrant and with the attached ciliary body and iris embed it in liver and cut sections through the *iridocorneal angle*. The *thick* sections are to be stained with Hansen's hematoxylin and mounted in damar (Fig. 258).

*b*. From the remaining three-fourths of the anterior half of the eye-ball cut out a piece of the cornea, 5 or 10 mm. square, embed it in liver and make sections through the *layers of the cornea* (Fig. 253). The alternating lamellæ of the substantia propria can only be well seen in unstained sections mounted in dilute glycerol.

*c.* From the posterior half of the eye-ball cut pieces including the three coats, 5 or 10 mm. square, and cut sections, not too thin, for the study of the *strata of the sclera and choroid* (Fig. 256). Stain them with Hansen's hematoxylin (p. 36) and mount in damar (p. 45). In sectioning, the retina usually becomes loosened.

*d.* For preparations showing the *entrance of the optic-nerve* cut around the point of entrance at a distance of about 5 mm. from the same through all the coats of the eye; embed this portion with about one centimeter of the optic-nerve in liver and cut sections (not too thin). Place the knife so that it strikes the retina first, then the choroid and sclera, and passes through the optic-nerve longitudinally; stain with dilute carmine (p. 36) and with Hansen's hematoxylin (p. 36), and mount in damar. Examine with very low magnification (Fig. 266).

No. 170.—Remove a fresh eye-ball according to the method given in No. 169, make an incision at the equator and place it in from 100 to 200 c.c. of Müller's fluid. In from twelve to twenty hours divide it with the scissors into an anterior and posterior half. In two or three weeks carefully wash both halves in slowly running water from one to two hours. Then cut pieces including all the coats about 8 mm. long and use for them the following preparations :—

*a. Teased Preparation of the Choroid.*—Tease and mount a fragment in a drop of dilute glycerol; it exhibits large blood-vessels, the capillaries of the choriocapillaris, branched pigment-cells, elastic fibers, sometimes also the glassy membrane; the "lattice-work" of the latter is only partially distinct. The isolated membranes may be stained with Hansen's hematoxylin and mounted in damar, but the more delicate structures are thus rendered indistinct (Fig. 257).

*b. Elements of the Retina.*—Carefully tease a small piece of the retina in a drop of Müller's fluid. Along with many fragments of the elements, a few more or less well-preserved parts will be found. Human eyes have very large, beautiful cone-visual cells, while those of many mammals are very small; wholly unsuitable in this respect are the eyes of the rabbit; unfortunately, human eyes are usually no longer in a sufficiently fresh condition when the investigation is made. The outer segments of the cones, also of the rods, are extremely delicate and rapidly disintegrate after death, falling into transverse plates and at the same time curving like a shepherd's crook. Later they disappear. In order to see beautiful cone-visual cells, examine, according to the method just given, the eyes of fishes. (See further, No. 173 and 174.)

*c.* The remaining parts of the eyeball are to be transferred from the water for hardening to 80 c.c. of gradually-strengthened alcohol (p. 33); when the hardening is completed, cut out the iris, embed it in liver, and make meridional sections; stain them in Hansen's hematoxylin (p. 36) and mount in damar (p. 45) (Fig. 259).

*d.* Cut out a portion 1 cm. long of the retina, including the *ora serrata*, which is macroscopically visible as a wavy line, embed it in liver,

and make meridional sections; stain them in hematoxylin (p. 36) and mount in damar (Fig. 265).

*e.* Treat in the same manner a piece of the *retina* taken from the posterior portion of the eye where the optic-fiber stratum is thickest. The radial fibers of Müller can only be seen in their entire length in accurate vertical sections (Fig. 261 and Fig. 265).

*f.* In the same manner treat meridional sections through the *macula* and *fovea.* It is not difficult to cut sections of the macula, but on the other hand very difficult to obtain satisfactory sections through the extremely delicate fovea. The retina should not be loosened from the choroid, but the two should be sectioned together. (Among the lower mammals only the ape possesses a yellow macula and a central fovea; on the other hand, the majority—insectivora and certain rodents excepted—have an "area centralis," without yellow pigmentation, but similar in structure to the macula. A simple or multiple fovea is always present in birds and reptiles; a fovea has also been found in bony fishes.)

No. 172.—*Retina, after Golgi.*—For this purpose *thick* retinæ are most suitable, therefore select the eyes of large animals. Divide the eye into an anterior and a posterior half, remove the vitreous body, and with forceps and scissors carefully dissect a piece of the retina from the choroid. Cautiously roll this piece into a cylindrical or spherical mass, and dip it for one second in thin celloidin-solution; expose it for a few seconds to the air, until the envelope of celloidin is somewhat stiffened, and then place the piece in the Golgi mixture (p. 41). (The object of this envelope is to prevent the formation of precipitates on the surface.) Let the object remain in the Golgi mixture for from twelve to seventy-two hours, then transfer it for twenty-four hours to the silver-solution (p. 41). Then repeat the procedure (p. 42). The impregnation occurs first, after twelve hours, in the rods and cones; after another twelve hours in the bipolar cells and "spongioblasts" (p. 351, remark), later in the cells of the ganglion nervi optici and in the nerve-fibers, last in the sustentacular cells.

No. 173.—*Fresh Elements of the Retina.*—Select the warm eyes of animals just killed. Divide the eyeball at the equator and carefully remove the vitreous body from the posterior half; cut small pieces about 3 mm. square from the transparent retina and tease in a drop of the vitreous humor; place two thin strips of paper on either side of the preparation (p. 48), and apply a cover-glass. Isolated elements will be found only here and there; on the other hand, very good surface views are not infrequently obtained in which the rods and cones are perceptible in optical cross-section, the former as small, the latter as large circles. If at the same time a little piece of the pigmented epithelium has been transferred to the slide, the regular hexagonal cells of the same can be plainly seen with the low power. The light spots in these cells are their nuclei (Fig. 8). These cells are very unstable and soon lose their sharp contours; molecular motion of the pigment-granules may be very frequently observed.

No. 174.—The best method for isolating the *elements of the retina* is the following: Place the eye unopened, but freed from fat and muscle, in 1 per cent. osmium solution. In twenty-four hours cut the eye open at the equator and place it for maceration for two or three days in distilled water; then with scissors cut out a piece of the retina about 2 mm. long and tease it in a drop of water; the preparation may be stained under the cover-glass with picrocarmine (p. 48) and mounted in dilute glycerol. With the high power, in addition to many fragments the source of which is not always to be determined with certainty, elements like those pictured in Fig. 263 may be found.

It is advisable to select the eyes of small animals—*e. g.*, a small water salamander—in which the sclera is thin and allows the osmium solution to penetrate easily. For such an eye 1 or 2 c.c. of the solution will be sufficient. The form of the rods is quite different from those of mammals; they are thick and are provided with long outer segments; the cones are small.

No. 175.—*Corneal Spaces and Canaliculi.*—Select an eye as fresh as possible; of the eyes of animals, that of the ox is most suitable; with the handle of a scalpel scrape away the epithelium of the cornea; spray the denuded surface with distilled water; cut through the eye in front of the attachment of the ocular muscles and place the anterior segment containing the entire cornea down on the epithelial side; then with forceps and scalpel remove the ciliary body, the lens, and the iris, so that only the anterior portion of the sclera and the cornea remain, which are to be placed in 40 c.c. of a 1 per cent. solution of silver nitrate. The whole is then placed in the dark for from three to six hours and then transferred to 50 c.c. of distilled water and exposed to sunlight (see further, p. 40). Harden the objects in 50 c.c. of gradually-strengthened alcohol and cut horizontal sections, which are most easily obtained if the cornea is held over the left index-finger. It is best to take the sections on the posterior surface of the cornea, since the spaces and canaliculi are more regular there. The sections may be stained in Hansen's hematoxylin and mounted in damar. The pictures are negative, the spaces and canaliculi white on a brown or brown-yellow surface (Fig. 254). Carefully examine the usually somewhat thinner margins of the section; in sections stained in hematoxylin the nuclei of the fixed corneal corpuscles are a dull blue; the contours of the cells can seldom be perceived.

No. 176.—*Fixed Corneal Corpuscles by the Gold Method.*—The method described on page 43 is to be somewhat modified, as follows: Express the juice from a fresh lemon; filter it through flannel. Kill the animal,* cut out the cornea and place it for five minutes in the lemon-juice, in which it becomes transparent; then wash it in 5 c.c. of distilled water for one minute; transfer it to 10 c.c. of gold-chlorid (p. 22) solution and place it in the dark for fifteen minutes. With glass rods transfer the cornea to

* Frogs are especially recommended; their corneal canaliculi are very regular and their posterior lamina is easily detached.

10 c.c. of distilled water for one minute, then to 50 c.c. of distilled water to which 2 drops of acetic acid have been added, and expose it to daylight; in from twenty-four to forty-eight hours the reduction is completed. The object is then to be placed in 10 c.c. of 70 per cent. alcohol (in the dark); on the following day cut out a little piece of the cornea, hold it with needle and scalpel at the edges and separate the thin lamellæ from the posterior surface; with a little attention this can be successfully done without much trouble. Mount the lamellæ in damar.

No. 177.—Very good preparations of the *corneal canaliculi* are obtained by the method of *Drasch*. The objects are not to be taken from the animal recently killed, but twelve or twenty-four hours after death, during which time the cadaver must be kept in a cool place. Small pieces of the cornea are to be cut out, about 6 mm. long, placed in 5 c.c. of 1 per cent. gold-chlorid solution plus 5 c.c. of distilled water, and stood in the dark for one hour; during this time frequently stir the fluid with a glass rod. With glass rods transfer the pieces to 30 c.c. of distilled water, in which they should remain (in the dark) for from eight to sixteen hours. They are then to be transferred to 25 c.c. of distilled water plus 5 c.c. of formic acid and exposed to daylight. When the reduction is completed (p. 43) the dark-violet pieces are to be hardened in gradually-strengthened alcohol and in about six days thin sections parallel to the surface can be cut and mounted in damar (Fig. 254).

No. 178.—*Nerves and Blood-vessels of the Fresh Cornea.*—Select the eye of an ox and cut out the cornea and the adjoining portion of the sclera extending from the limbus to the attachment of the ocular muscles; with scalpel and forceps remove the ciliary body, iris, and lens, immediately cut out a quadrant of the cornea, place it with the epithelial side up on a slide and apply a cover-glass; a drop of the vitreous humor may be added. The very thick preparation must be examined with a low power. When the surface of the cornea is in focus the loop-shaped blood-vessels can be seen at the scleral margin; the majority still contain blood-corpuscles. Medullated nerve-fibers are found here, as well as in the deeper strata; they are arranged in bundles and within the cornea can only be traced for a short distance. The elongated pigment-streaks found in the eye of the ox have no relation to the nerves.

This method is not serviceable for the exhibition of the finer distribution of the nerves.

No. 179.—*Nerves of the Cornea.—a. Gold Method.*—Cut out the cornea twelve or twenty-four hours after death, remove the ciliary body and iris, and treat it according to the method given in No. 176. When the hardening is completed cut horizontal sections, which contain the epithelium and the uppermost strata of the cornea, and vertical sections through the thickness of the cornea. Mount in damar (Fig. 270).

*b. Methylene Blue Staining.*—Kill a rabbit; remove the entire eyeball, free it from the attached remnants of ocular muscles and connective-tissue, place it in a watch-glass and with a sharp scalpel make a deep

incision through all the coats of the eye at the equator; thus the vitreous humor escapes into the watch-glass. Then with scissors separate from the point of incision the entire cornea, place it on a slide with the concave surface upward and with the handle of the scalpel scrape off the ciliary body, iris, and lens, which is easily done; transfer the cornea thus cleansed to a second watch-glass containing from 3 to 10 drops of the vitreous humor and from 3 to 4 drops of a $\frac{1}{15}$ per cent. methylene blue solution (p. 25). The concave surface of the cornea should be uppermost and covered by the staining fluid.

The time required for staining cannot be given with certainty; therefore it is advisable after several hours to place the cornea with the *convex* surface up on a clean slide and, without a cover-glass, to examine it with the low power; if it is not sufficiently stained return it to the watch-glass and examine it again in about ten minutes.

So soon as the nerves can be distinctly seen the cornea is to be transferred for from eighteen to twenty hours to 20 c.c. of the ammonia solution (p. 39); then cut out a quadrant and mount it in dilute glycerol, to which a drop of the ammonia-solution has been added; after being kept in the dark for twenty-four hours the preparation is sufficiently transparent and can be investigated with the high power.

No. 180.—*Lens-fibers.*—Cut the eye-ball open back of the equator; remove the vitreous body and lens; thus the pigment covering the ciliary processes remains attached to the margin of the lens. Loosen the lens from the vitreous body and place it in 50 c.c. of Ranvier's alcohol (p. 20). In about two hours thrust needles into the anterior and posterior surfaces of the lens and strip the capsule up from a small area; this is easily done; if lens-fibers are attached to the capsule it does not matter. On pricking the lens a turbid white fluid escapes; shake the alcohol and let the lens remain in it for from ten to forty hours. At the expiration of this time the lens can be easily separated into shell-like pieces. Tease a small strip of one of these pieces in a small drop of salt solution on a slide (p. 19). Apply a cover-glass, taking care to avoid pressure; if it is desired to preserve the fibers, stain with picrocarmine (staining usually occurs in a few minutes), and mount in dilute acidulated glycerol (Fig. 267, *A*).

No. 181.—*Lens-fibers in Transverse Section.*—Place a lens in 50 c.c. of 0.05 per cent. chromic acid. A cloth or a little cotton must be placed on the bottom of the bottle or the lens will adhere to the glass and burst. This may also be prevented by frequently shaking the bottle. In from twenty-four to forty-eight hours with a needle break the lens into shell-like pieces, transfer them after ten or fifteen hours to 30 c.c. of 70 per cent. alcohol, which is to be replaced on the following day by an equal quantity of 90 per cent. alcohol. With the scissors cut the pieces through in the region of the equator, and so embed them in liver that the first sections will pass through the zone lying next to the equator. If the section, which need not be very thin, has passed through the fibers transversely they will appear as sharply-defined hexagons; if, on the contrary, the

section is oblique, the single fibers will appear to be separated from one another by irregular zigzag lines; they may even be cut partially lengthwise. The sections are to be transferred directly from the blade to the slide and mounted in dilute glycerol (Fig. 267, *B*).

No. 182.—*The Lens Capsule and the Lens Epithelium.*—Place the eyeball, free from muscle and fat, in 100 or 200 c.c. of Müller's fluid. Treat it further as follows:

*a. Surface View of the Lens Capsule and Epithelium.*—After two or three days cut the eye open, remove the lens, and with forceps strip off a piece of the anterior lens capsule; place it for about five minutes in a watch-glass with distilled water, which is to be changed once, then stain it in Hansen's hematoxylin; mount in damar. The capsule is stained a homogeneous light blue; the nuclei and contours of the epithelial-cells are very sharp (Fig. 268, *C*). If it is desired to obtain the lens capsule alone strip off a portion of the posterior lens capsule.

*b. Sections of the Capsule and Epithelium.*—Let the eye-ball remain in Müller's fluid for two weeks; remove the lens, wash it for one hour in running water and harden it in 50 c.c. of gradually-strengthened alcohol (p. 33); cut meridional sections through the anterior surface and the equator of the lens; stain them with Hansen's hematoxylin (p. 36) and mount in damar (Fig. 268, *D*).

No. 183.—*The Blood-vessels of the Eye.*—For this purpose surface preparations are especially suitable. Open a fresh eye at the equator. The course of the central artery of the retina is macroscopically perceptible. For the exhibition of the blood-vessels of the choroid place an eyeball completely freed from attached muscle and fat on a small glass funnel, which has been thrust into a low glass bottle, and with scissors and forceps, beginning at the equator, carefully dissect off the sclera. With a little practice the entire sclera can be removed beyond the ora serrata up to the optic entrance without injury to the choroid; care must be taken not to tear it. (Beginners should be content to remove only one quadrant of the sclera.) All the firmer points of attachment between the sclera and choroid (the venæ vorticosæ) must be cut through. Then by careful brushing with a sable pencil moistened in water remove the attached portions of the lamina suprachoroidea from the choroid; by this manipulation the course of the larger blood-vessels is brought to view. So far the investigation may be pursued on the uninjected eye (compare with No. 170, *a*). For the study of the blood-vessels of the ciliary body and the iris it is necessary to use an injected eye, divided anterior to the equator, fixed in Müller's fluid and hardened in alcohol. The iris and ciliary body may be easily stripped from the sclera; remove the lens and mount in damar. Examine at first with the low power.

No. 184.—Place the *upper eyelid* of a child in 100 c.c. of 0.5 chromic acid for from one to three days, wash it two hours in running water, and harden in 50 c.c. of gradually-strengthened alcohol. For a general view cut

thick (Fig. 271), for the finer details thin sections (Fig. 25, *C*). Staining with Hansen's hematoxylin is at first difficult, but more readily accomplished after the object has lain in alcohol several months (compare p. 36, remark *). Mount in damar.

No. 185.—*The Lacrymal Glands.*—The lower *tear-gland* in man can be easily removed, without visible external injury, from the fornix of the conjunctiva. In the rabbit this gland is very small and when fresh resembles pale muscle tissue. It must not be confused with Harder's gland lying in the median angle of the eye. Treat like No. 112. Small pieces 1 mm. square can be used. The excretory duct and tubules may be easily seen; difficult, on the other hand, it is to see the intercalated tubules, the epithelium of which differs greatly in height and occasionally is so low that care must be taken not to confuse them with blood-vessels (Fig. 272).

# XII. THE ORGAN OF HEARING.

The organ of hearing consists of three divisions ; the innermost, the *internal ear*, encloses the end-apparatus of the auditory nerve ; the other divisions, the *middle ear* and the *external ear*, are only accessory apparatus.

## THE INTERNAL EAR.

The internal ear consists of two membranous saccules lying within the bony vestibule (vestibulum), that communicate with each other by means of a minute canal, the *ductus utriculo-sacculari*s. The one saccule, the *utricle*, is in connection with membranous tubules, the *semicircular canals* (ductus semicirculares), each of which at the point where it opens in the utricle possesses a dilatation, the *ampulla*. The other saccule, the *sacculus*, connects by means of the *ductus reuniens* with a long spirally-wound membranous sack, the *cochlea* (ductus cochlearis).

The sacculus and utriculus, the semicircular canals and the cochlea are called the *membranous labyrinth*. This is enclosed within the petrous bone in a space having similar outlines, the *bony labyrinth*, which it does not completely fill. The unfilled space is occupied by a watery fluid, the *perilymph*. A similar fluid, the *endolymph*, is contained within the interior of the membranous labyrinth.

The saccules and the semicircular canals exhibit the same structure, but the cochlea is so essentially different that it requires a separate description.

### The Saccule, the Utricle, and the Semicircular Canals.

The walls of these canals comprise three layers. The outermost is a connective-tissue layer rich in elastic fibers ; this is followed within by a delicate basal membrane beset with minute excrescences, which on its inner surface is covered by a simple squamous epithelium. This simple structure undergoes alteration at the positions where the filaments of the auditory nerve are distributed, the *maculæ cribrosæ* of the saccule and utricle, the *cristæ acusticæ* on the ampullæ of the semicircular canals. The connective tissue and basal membrane here become thicker ; the squamous epithelium already in the vicinity of the maculæ and cristæ

becomes transformed into a columnar epithelium with a cuticular border, and this passes into the neuro-epithelium of the maculæ and cristæ. The neuro-epithelium likewise is a simple layer and consists of two kinds of cells: (1) *fiber-cells*, elongated elements occupying the entire depth of the epithelium, slightly expanded at the upper as well as at the lower end, and containing an oval nucleus; they are the sustentacular elements; (2) *hair-cells*, cylindrical elements occupying only the upper half of the epithelium, which in their lower rounded division contain a large spherical nucleus and bear on their free surface a bundle of long, delicate agglutinated filaments, the "auditory hairs." The hair-cells are the terminal apparatus of the auditory nerve. The nerve-fibers of the ramus vestibularis nervi acustici are in connection with the hair-cells and in this way. On entering the epithelium the nerve-fibers lose their medullary sheath, divide, and as naked axis-cylinders ascend to the base of the hair-cells; there each fiber divides into three or four varicose twigs, that run beneath several hair-cells parallel to the surface of the epithelium, and finally turn upward and terminate in contact with the lateral surface of a hair-cell in a free pointed end.* During their horizontal course they send upward a few twigs, that in the same manner end in contact with the hair-cells. These ends do not reach to the surface of the epithelium. The free surface of the neuro-epithelium is covered by a continuation of the cuticular zone, a "limitans," which is perforated by the auditory hairs. The maculæ acusticæ are covered by a soft substance (a cuticula?), in which innumerable prismatic crystals of calcium carbonate, *the otoliths*, from 1 to 15 $\mu$ in size, are embedded; they form the "otoconia," the auditory sand. On the cristæ acusticæ the so-called *cupula* occurs, in fresh preparations an invisible substance, that on the application of fixation fluids coagulates and thus becomes visible.

FIG. 273.—OTOLITHS FROM THE SACCULUS OF AN INFANT. × 560. Techn. No. 186.

By means of strands of connective tissue the saccules and the semicircular canals are secured to the bony labyrinth, the inner surface of which is covered by a thin periosteum and flattened connective-tissue cells.

### THE COCHLEA.

The membranous cochlea, the *ductus cochlearis*, does not entirely fill the space within the bony cochlea. It lies with one wall in contact with

* The horizontal branches interlace and form a small, but dense "lattice-work," that also by other methods than that of Golgi appears to consist of a special layer of strongly-refracting granules. The granules are the varicosities and the optical cross-sections of the horizontal fibers.

the outer wall * of the bony cochlea (Fig. 274); the upper or vestibular wall, the *vestibular membrane* (Reissner), bounds the scala vestibuli; the lower or tympanic wall, the *membranous spiral lamina*, is directed toward the scala tympani. The angle in which the vestibular and tympanic wall meet lies on the free end of the osseous spiral lamina. There the periosteum and the connective tissue of the ductus cochlearis are especially well developed and form a prominence, the *limbus spiralis*, which rests with a broad surface on the bony spiral lamina, slopes upward and terminates in a sharp edge. This edge is called the *labium vestibulare*, the free margin of the bony spiral lamina is called the *labium*

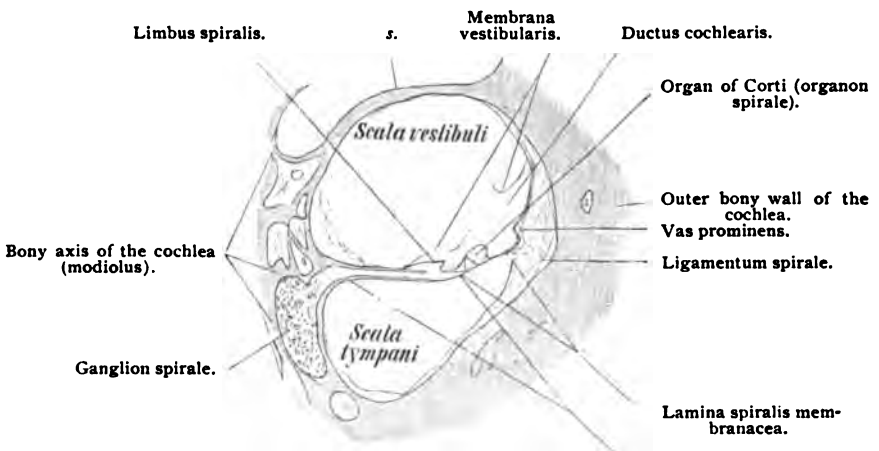


FIG. 274.—SECTION THROUGH THE SECOND TURN OF THE COCHLEA OF AN INFANT. × 25. The modiolus contains longitudinal canals cut obliquely. *s*. Bony wall between the second and third (half) turns of the cochlea. The membrana vestibularis is torn through, the upper fragment being turned upwards. The membrana tectoria could not be seen. Techn. No. 188.

*tympanicum*, between the two runs the *sulcus spiralis* (Fig. 280). The inner surfaces of the ductus cochlearis are covered by an epithelium that varies greatly in different localities; the outer surfaces, toward the scala vestibuli and scala tympani, are covered by a delicate continuation of the periosteum which clothes both scalæ. On the outer wall of the cochlea the periosteum becomes greatly thickened and in cross-section appears as a crescentic mass, the *ligamentum spirale*, that extends above and below the attached surface of the ductus cochlearis (Fig. 274).

The minute structure of the outer and the vestibular wall of the

* I here follow the customary description, in which the cochlea is placed in such a manner that the base is directed downward, the summit upward; accordingly, "inner" is toward the axis of the cochlea, "outer" toward the periphery.

membranous cochlea is comparatively simple, that of the tympanic wall, on the other hand, is extremely complicated.

The *outer wall* and the *spiral ligament* together consist of epithelium and connective tissue. The latter, next to the bone, is a dense fibrous tissue (the periosteum); this passes into a loose connective tissue which contributes the chief bulk of the spiral ligament. The epithelium consists of a row of cubical epithelial cells. A dense network of blood-vessels, the *stria vascularis*, occupies three-fourths of the height of the outer cochlear wall, and at its lower end is limited by a vein that projects farther into the lumen of the cochlea, the *prominentia spiralis* (vas prominens) (Fig. 274). The capillaries of the stria vascularis lie close beneath the epithelium; they are the source of the endolymph.

The *vestibular wall*, membrana vestibularis (Reissner) (Fig. 274), consists of a process of the periosteum of the scala vestibuli, that is, of delicate fibrous connective tissue and flattened cells, which on the surface turned toward the ductus cochlearis is clothed with a simple layer of polygonal epithelial cells.

The *tympanic wall* consists of two portions, (1) the *limbus*, with the free margin of the bony spiral lamina, and (2) the *lamina spiralis membranacea*.

The *limbus* consists of compact connective tissue, containing an abundance of spindle-shaped cells, which below is continuous with the

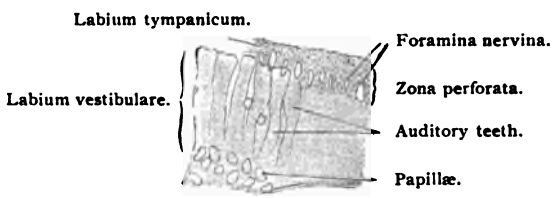


FIG. 275.—A SURFACE VIEW OF THE LAMINA SPIRALIS OF A CAT. × 240. The vestibular lamina is seen from above; between the auditory teeth two nuclei of the epithelial-cells are seen. On the left of the picture the plane of the auditory teeth is in focus, on the right, the plane of the zona perforata. Techn. No. 187.

tissue of the periosteum, on its free surface is beset with peculiarly-shaped papillæ. They have the form of an irregular hemisphere; toward the labium vestibulare they develop into small, elongated plates, the so-called *auditory teeth* (Fig. 275 and Fig. 278), that lie in a single row beside one another. The surface of the limbus is covered by a simple layer of flattened epithelial cells, which at the edge of the labium vestibulare passes into the cubical epithelium of the sulcus spiralis (Fig. 278, *A*).

The upper surface of the free margin of the osseous spiral lamina is

perforated by a single row of slit-like openings, the *foramina nervina* (Fig. 275), through which the nerves enclosed within the bony lamina emerge, to penetrate within the epithelium of the lamina spiralis membranacea. This portion of the osseous spiral lamina is called *zona perforata.*

The *membranous spiral lamina* (lamina spiralis membranacea) consists of (1) the *membrana basilaris*, an extension of the limbus and of the periosteum of the osseous spiral lamina, (2) the *tympanic lamella*, a process of the periosteum of the scala tympani which clothes the lower surface of the basilar membrane, and (3) the *epithelium of the ductus cochlearis*, which rests upon the upper surface of the basilar membrane.

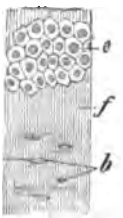


FIG. 276.—SURFACE VIEW OF THE LAMINA SPIRALIS MEMBRANACEA OF A CAT. × 240. Strata of the zona pectinata drawn with change of focus. *e*. Indifferent epithelium (cells of Claudius) of the ductus cochlearis in focus; *f*, the fibers of the membrana basilaris in focus; *b*, the nuclei of the tympanic lamella in focus. Techn. No. 185.

The *membrana basilaris* consists of a structureless substance which contains rigid, perfectly straight fibers, extending from the labium tympanicum to the spiral ligament, and of oblong nuclei. The membrane has a finely-striated appearance (Fig. 276, *f*).

The *tympanic lamella* consists of a delicate connective tissue containing spindle-cells, the fibers of which are disposed vertically to the elements of the basilar membrane (Fig. 276, *b*).

The *epithelium* of that half of the membranous spiral lamina toward

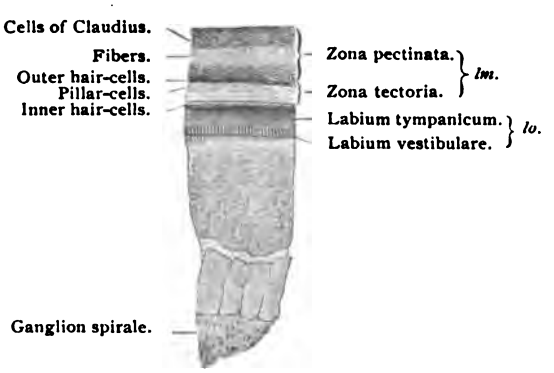


FIG. 277.—LAMINA SPIRALIS OF A CAT SEEN FROM THE VESTIBULAR SURFACE. The membrana tectoria has been removed. × 50. *lo*. Lamina spiralis ossea, inner half cracked and broken at several points; at the posterior border of the same cells of the spiral ganglion project forth. *lm*. Lamina spiralis membranacea. The cells of Claudius have partly fallen off, so that the fibers of the membrana basilaris are seen as a delicate striation. Techn. No. 187.

the axis of the cochlea is differentiated as the highly-specialized neuro-epithelium of the *spiral organ* (organ of Corti), while that occupying the outer half, toward the spiral ligament, consists of indifferent epithelial

elements. Therefore the spiral lamina is divided into two zones: an inner, occupied by the spiral organ, *zona tecta*, and an outer, *zona pectinata*, so called because of the striations of the basilar membrane shimmering through it.

The most remarkable elements of the spiral organ are the *pillar-cells*, peculiarly-shaped, for the greater part rigid forms, arranged in two rows the entire length of the cochlea. The inner row of pillar-cells form the *inner pillars*, the outer row, the *outer pillars* (Fig. 278). The two

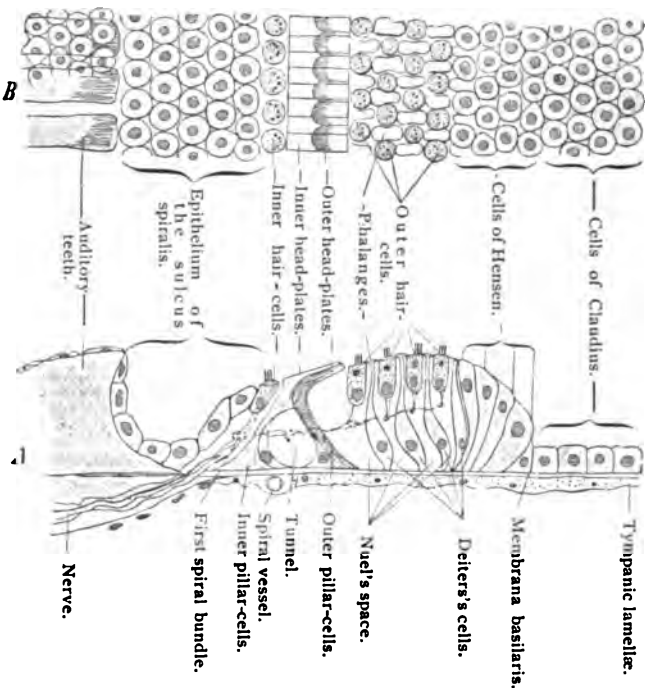


FIG. 278.—SCHEME OF THE STRUCTURE OF THE TYMPANIC WALL OF THE DUCT OF THE COCHLEA. *A*. Seen from the side. *B*. Seen from the surface. In the latter, the free surface is in focus. It is evident that the epithelium of the sulcus spiralis, lying in another plane, as well as the cells of Claudius, can only be distinctly shown by depressing the tube. The membrana tectoria is not drawn. The spiral nerve-bundles are indicated by dots.

rows of pillars are obliquely inclined toward one another and form an arch, the *arcus spiralis*, which spans a triangular space, the *tunnel*, the base of which is directed toward the basilar membrane. The tunnel is nothing more than a very large intercellular space, that is filled with a soft mass, the intercellular substance. Regarding the histology of the pillar-cells, the following details are to be considered: The *inner pillar-cells* are rigid bands, in which a *three-sided expanded foot*, a *slender body*, and a *concave head*, with the concavity directed outward, may be

distinguished. The head is furnished with a thin process, the "head-plate" (Fig. 278). The body and foot of the cell are surrounded by a scant amount of protoplasm, that only to the outer side of the foot in the vicinity of the nucleus is present in somewhat larger amount. The *outer pillar-cells* exhibit the same details, excepting that the portion containing the nucleus lies to the inner side of the foot; the rounded articular head rests in the concave facet of the head of the inner pillar-cells, the broader head-plate is covered in its greater part by the head-plate of the inner pillars. To the inner side of the inner pillars lies a *simple* row of cells, the *inner hair-cells*, short cylindrical elements that do not extend to the basilar membrane; they possess a rounded base and about forty stiff hairs on their free surface. To the inner side of the inner hair-cells lies the cubical epithelium of the sulcus spiralis. On the outer side of the outer pillars lie the *outer hair-cells;* they resemble the inner hair-cells, but possess hairs that are one-third shorter and are char-

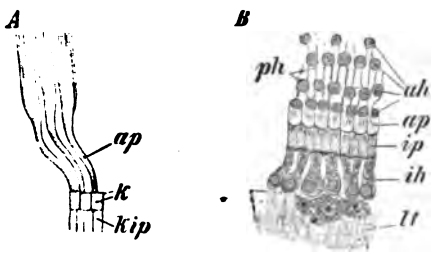


FIG. 279.—SURFACE VIEW OF THE LAMINA SPIRALIS MEMBRANACEA OF A CAT. × 240. *A*. Outer pillar-cells; *k*, head-plates of the same, upper surface in focus; *ap*, body and inferior extremity drawn with gradual depression of the tube; *kip*, portions of the head-plates of the inner pillar-cells. *B. lt.* Labium tympanicum partly covered by the epithelium of the sulcus spiralis; *ih*, inner, *ah*, outer hair-cells, between these the phalanges, *ph*, forming the membrana reticularis; *ap*, head-plates of the outer, *ip*, of the inner pillar-cells. Techn. No. 187.

acterized by a dark body occupying the upper half of the cell, the *spiral body.** The outer hair-cells are arranged in several (usually four) rows; they do not lie in contact with one another, but are held apart by *Deiters's cells;* these are elongated cells that contain a rigid filament and at their upper end support a *cuticular top-plate*, which has the form of a digital phalanx. The free spaces between the "phalanges" are occupied by the upper ends of the outer hair-cells† (Fig. 279). The cells of Deiters are sustentacular elements, that exhibit much in common with the pillar-cells; like these they consist of a rigid filament and a proto-

* In the scheme (Fig. 278, *A*) this body is indicated by a dark speck close beneath the auditory hairs.

† The inner hair-cells are kept apart from one another by short processes of the inner pillar-cells. These processes are not shown in Fig. 278, *B*.

plasmic portion, like these they have a head-plate (named phalanx). The difference consists only in this, that the transformation into rigid parts is not so far advanced in the cells of Deiters. The phalanges are joined to one another and form a beautiful netted membrane, the *membrana reticularis.*

The outer hair-cells do not extend to the basilar membrane, but occupy only the upper half of the spaces between the cells of Deiters; the lower divisions of these spaces remain unoccupied, and are called *Nuel's spaces* or, since they communicate with one another, the space of Nuel (Fig. 278, *A*). The latter also has the significance of an intercellular space and is connected with the tunnel.

External to the last row of Deiters's cells lie the cells of Hensen,

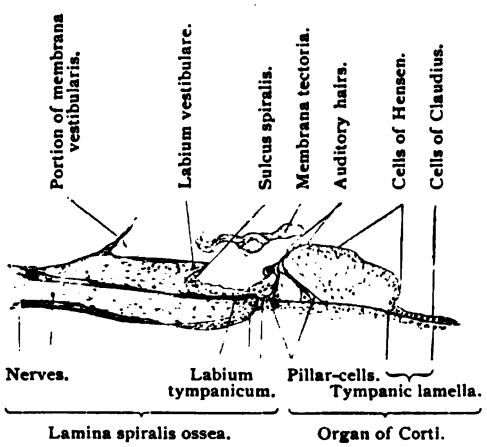


FIG. 280.—VERTICAL RADIAL SECTION THROUGH THE PERIPHERAL HALF OF THE LAMINA SPIRALIS OSSEA AND THROUGH THE LAMINA SPIRALIS MEMBRANACEA OF AN INFANT. × 80. The membrana tectoria has been torn from its point of attachment on the labium vestibulare. Techn. No. 188.

elongated cylinders, that gradually decrease in height and pass into the indifferent epithelium of the cochlear duct, the elements of which, so far as they cover the basilar membrane, are called the *cells of Claudius.*

A soft, elastic cuticular formation, the *membrana tectoria*, lies above the sulcus spiralis and the spiral organ (Fig. 280). It is attached to the vestibular lip of the sulcus and extends to the outermost row of hair-cells.

The *cochlear branch* of the *auditory nerve* penetrates into the axis of the cochlea and in its spiral uninterrupted ascent gives off branches which pass to the root of the osseous spiral lamina; here each medullated nerve-fiber loses its medullated sheath and passes into a nerve-cell that like those of the spinal ganglia possesses a connective-tissue capsule;

these nerve-cells collectively form the *ganglion spirale*,* which winds along the entire periphery of the axis of the cochlea (Fig. 274); from the opposite pole of each cell springs a second nerve-fiber, that soon acquires a medullated sheath and unites with neighboring fibers in a wide-meshed plexus enclosed within the osseous spiral lamina; this plexus extends near to the labium tympanicum, where the fibers lose their medullated sheath, escape through the foramina nervina and end in the epithelium. This occurs in such a manner that they bend in the direction of the circumvolution of the cochlea and run in spiral bundles, of which the first passes to the inner side of the inner pillar-cells, the second into the tunnel, the third between the outer pillar-cells and the first row of the cells of Deiters, the remaining three between the cells of Deiters. From these bundles delicate fibers proceed to the hair-cells, on which (not within) they terminate.

*The Arteries of the Labyrinth.*—The auditory artery gives only a small twig to the membranous labyrinth and another small twig to the bony labyrinth; the majority of its branches pass to the roots of the fifth, seventh, eighth, ninth, and tenth cranial nerves and to the under surface of the cerebellum. The artery for the membranous labyrinth divides into two branches: 1. The *arteria vestibularis* (Fig. 281) sends twigs to the vestibular nerve and to the lateral upper half of the sacculus and utriculus, as well as to the corresponding portions of the upper and lateral semicircular canal, which supply a capillary plexus that in general is wide-meshed, but at the terminal points of the vestibular nerve, the cristæ and maculæ, is narrow-meshed. 2. The *arteria cochlearis communis* divides in two branches. The one branch, the *arteria vestibulo-cochlearis*, supplies one twig to the median-posterior half of the sacculus, utriculus, and semicircular canals and in its minute ramifications behaves like the vestibular artery; another twig ramifies in the initial third of the first turn of the cochlea. The other branch, the *arteria cochlearis propria*, supplies the remaining extent of the cochlea; on entering the axis of the cochlea it divides into three or four branches, which in their spiral ascent form the tractus arteriosus spiralis. From this about 30 or 35 radial twigs arise, which supply three separate capillary territories: (1) the canal in which the ganglion spirale is enclosed, (2) the lamina spiralis, (3) the intermediate and outer walls of the scalæ (Fig. 282, 1, 2, 3).

* The ganglion spirale possesses the same structure as the spinal ganglia, with a single difference,—the ganglion-cells are not unipolar, but bipolar, as in the embryonal ganglia. The ganglion vestibulare in the interior of the cochlea also possesses bipolar ganglion-cells.

The *veins* of the labyrinth follow three separate paths:

1. The *vena aquæductus vestibuli* runs through the aquæductus vestibuli; it collects the blood from the semicircular canals and from one portion of the utriculus; it opens in the sinus pretrosus superior (Fig. 281).

2. The *vena aquæductus cochleæ* runs through the aquæductus

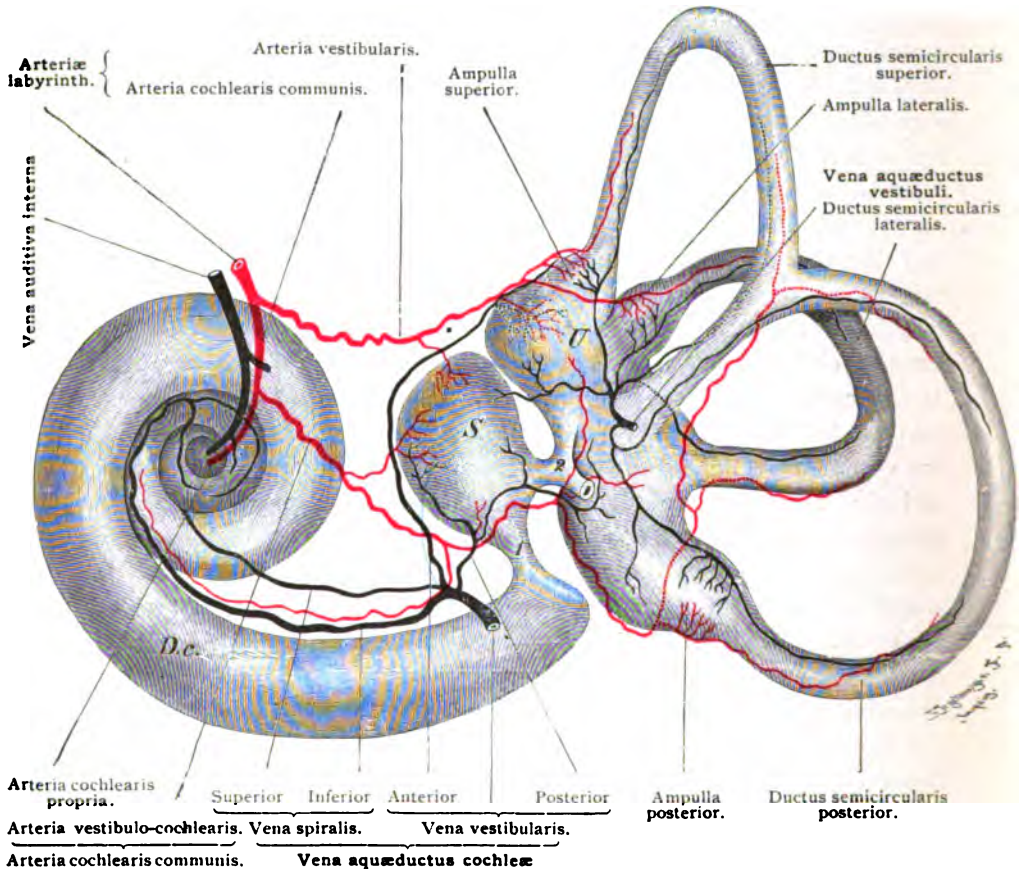


FIG. 281.—SCHEME. BLOOD-VESSELS OF THE RIGHT HUMAN LABYRINTH. MEDIAN AND POSTERIOR ASPECT. *D. c.* Ductus cochlearis. *S.* Sacculus. *U.* Utriculus. 1. Ductus reuniens. 2. Ductus utriculo-sacculari. The saccus endolymphaticus is cut off.

cochleæ; it collects the blood from one portion of the utriculus, from the sacculus and from the cochlea. The venous radicles in the cochlea behave in the following manner: The veins collecting at the *vas prominens* and at the *vas spirale* (Fig. 282, *a*, *b*) pass in the wall of the scala tympani to the spirally-running *vena spiralis*, lying below the spiral ganglion; this originates from the confluence of two veins, of which the

lower receives the blood from the first (basal) and a portion of the second turn of the cochlea, while the upper spiral vein collects the blood from the remaining cochlear turns. The spiral vein also takes up one set of the capillaries in the canal of the spiral ganglion and is united by anastomosis with a vein lying above this canal, the *vena lamina spiralis* (Fig. 282). This receives the blood from the other set of capillaries of

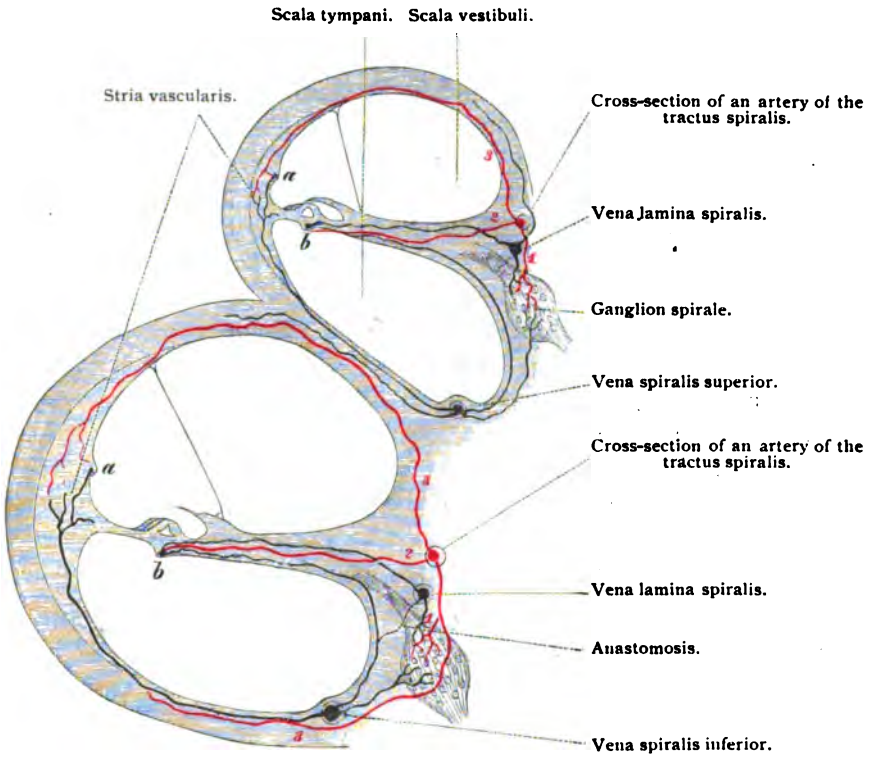


FIG. 282.—SCHEME. VERTICAL SECTION OF THE RIGHT HALF OF THE FIRST (BASAL) AND SECOND TURNS OF THE COCHLEA. *a*. Vas prominens. *b*. Vas spirale.

the spiral ganglion, as well as from the lamina spiralis,* and opens in the central vein of the cochlea.

3. The *central vein of the cochlea* is the main radicle of the internal auditory vein. The latter takes up veins from the auditory nerve and from the bone, and in all probability opens in the vena spinalis anterior.

*The Lymph-channels.*—The endolymph in the interior of the mem-

* The vestibular membrane is nonvascular in the adult. The arrangement of the blood-vessels in the cochlea is such that the scala vestibuli is chiefly encircled by arteries, the scali tympani mainly by veins. The portion of the scala tympani adjacent to the lamina spiralis membranacea is thus removed from the influence of arterial pulsation.

branous labyrinth is connected with the subdural lymph-spaces by means of minute canals passing from the ductus endolymphaticus. The perilymphatic spaces (see p. 185) are in connection with the subarachnoidal space by means of a lymph-vessel running through the aquæductus cochleæ, the "ductus perilymphaticus." Blood-vessels and nerves are surrounded by conspicuous perivascular and perineural lymph-spaces, that probably also are connected with the subarachnoidal space.

## The Middle Ear.

The *mucous membrane of the tympanic cavity* is intimately united with the underlying periosteum. It consists of thin connective tissue and a single stratum of cubical epithelial-cells, that sometimes on the floor, occasionally also in larger areas of the tympanic cavity, are ciliated. Glands (short, 0.1 mm. long follicles) occur sparingly in the anterior half of the tympanic cavity. The *mucosa of the eustachian tube* consists of a fibrillar connective tissue (containing numerous leucocytes near the pharyngeal orifice) and of a stratified ciliated columnar epithelium; the ciliary wave is directed toward the pharynx. Mucous glands occur in especial abundance in the pharyngeal half of the tube. The cartilage of the eustachian tube, where it adjoins the bony tube, is of the hyaline variety and here and there contains rigid (not elastic) fibers (cf. p. 83); in the anterior portion the matrix of the cartilage is penetrated by dense networks of elastic fibers. The *blood-vessels* in the mucosa of the tympanic cavity form a wide-meshed, in the mucosa of the eustachian tube a narrow-meshed superficial capillary network and a deep capillary plexus surrounding the glands. The *lymph-vessels* run in the periosteum of the tympanic cavity. With regard to the terminations of the nerves, exact information is still wanting.

## The External Ear.

The *tympanum* consists of a lamina of connective tissue, *lamina propria*, in which the fibrous bundles on the outer side are radially arranged and connected with the periosteum of the sulcus tympanicus, while on the inner side, toward the tympanic cavity, the fibrous bundles are circularly arranged. On its inner surface the tympanum is covered by the mucous membrane of the tympanic cavity, on its outer surface by the integument of the external auditory canal. Both investments are very firmly attached to the lamina propria, are smooth, and are without papillæ. Where the malleus lies against the tympanum, the latter is provided with a superficial stratum of hyaline cartilage.

The *external auditory canal*, so far as it is cartilaginous and on the

whole length of its upper wall, is clothed with an extension of the skin, which is characterized by its thickness and by a great abundance of peculiar coil-glands, the *ceruminous glands*. In some respects these glands correspond with the ordinary larger coil-glands (sweat-glands) of the skin; like these, they possess an excretory duct lined by several layers of epithelial-cells, and the tubules of the coil contain a simple layer of cubical gland-cells, which rest on smooth muscle-fibers and a conspicuous basement membrane (Fig. 284); they are distinguished from the sweat-glands by the very wide lumen of the coiled tubule, that particularly in adults is greatly dilated, and by numerous pigment-granules and fat-droplets within the gland-cells, which frequently exhibit a distinct

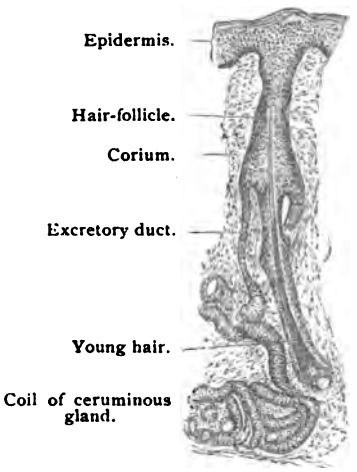


FIG. 283.—FROM A VERTICAL SECTION THROUGH THE SKIN OF THE EXTERNAL AUDITORY MEATUS OF AN INFANT. × 50. The excretory duct opens into the hair-follicle. Techn. No. 191.

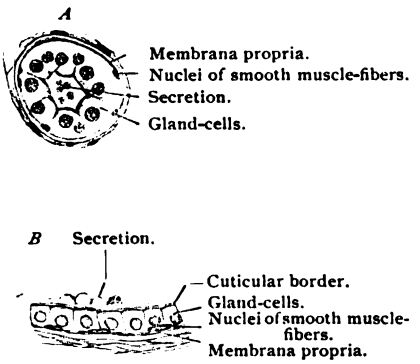


FIG. 284.—*A*. CROSS-SECTION OF A COIL-TUBULE OF THE SKIN OF THE EXTERNAL AUDITORY MEATUS OF AN INFANT. *B*. LONGITUDINAL SECTION OF A COIL-TUBULE FROM THE EXTERNAL AUDITORY MEATUS OF A TWELVE-YEAR-OLD BOY. × 240. Techn. No. 191.

cuticular border. The excretory ducts are narrow and in children open in the hair-follicles, in adults, close beside the hair-follicles on the free surface. The secretion, the *cerumen*, consists of pigment-granules, oil-globules, and cells containing fat; the latter probably come from the sebaceous glands of the hair-follicles. In the (remaining) region of the bony external auditory meatus, the integument is thin and without ceruminous glands.

The cartilage of the external auditory canal and of the pinna is of the yellow elastic variety.

The *blood-vessels* and *nerves* are distributed as elsewhere in the skin; only on the tympanum do they exhibit peculiarities. Close behind the

handle of the malleus an artery descends, which breaks up into radially-disposed branches; the blood is returned by two paths: (1) by a venous plexus extending along the handle of the malleus and (2) by a venous plexus lying on the margin of the tympanum.

These vessels lie in the integumentary covering of the tympanum. The mucous membrane of the tympanum is provided with a dense capillary network, which anastomoses with the integumentary vascular network by means of perforating branches at the margin of the tympanum.

The *lymph-vessels* are principally found in the cutaneous stratum of the tympanum.

The *nerves* form delicate networks lying beneath the mucous and the cutaneous covers.

## TECHNIC.

A fundamental condition is an exact knowledge of the macroscopic anatomy of the labyrinth. The difficulties, the failures, depend in the main on inaccurate knowledge of the bony labyrinth. As a preliminary all parts lying lateral to the promontory (os tympanicum and ossicles of the ear) must be removed, so that this is distinctly visible.

No. 186.—*Otoliths.*—Chisel out the promontory, beginning at the upper margin of the fenestra stapedii, to the lower margin of the fenestra rotunda. Then, especially if the bone is placed in water, the white spots (maculæ) in the sacculus and utriculus can be detected. With delicate forceps lift out the saccules and spread out a small piece in diluted glycerol on a slide. The otoliths are present in large numbers, but are very small, so that their shape can only be distinctly seen with the high power (240 diameters). The glycerol must not be too thick, or it will render the otoliths completely invisible (Fig. 273).

In taking out the saccules portions of the semicircular canals are not infrequently also removed; stain these with picrocarmine and mount them in dilute glycerol. Only the epithelium and here and there in optical section the delicate glassy membrane can be seen. The connective tissue is scanty.

No. 187.—*The Cochlea.*—The base of the cochlea lies in the bottom of the internal auditory meatus, the apex is directed toward the eustachian tube, therefore the axis of the cochlea is horizontal and transverse to the long axis of the petrous bone.

Open the free portion of the cochlea, that is, remove the promontory close to the fenestra rotunda, open the apex of the cochlea and having removed the superfluous osseous mass as far as practicable place the preparation in 20 c.c. of 0.5 per cent. osmic acid (5 c.c. of 2 per cent. osmic acid to 15 c.c. of distilled water). In from twelve to twenty hours wash the preparation for about one hour and then place it in 200 c.c. of Müller's fluid. In from three to twenty days (or later) open the cochlea

and examine it in water. The osseous spiral lamina can be seen as a delicate lamella, the membranous spiral lamina as a delicate membrane, attached to the axis of the cochlea; with fine forceps break off pieces of the osseous spiral lamina; do not lift them with the forceps, but carefully with needle and section lifter remove them from the fluid and transfer them to a drop of dilute glycerol on a slide. It is advisable to break off the axial portion of the spiral lamina on the slide with needles, because the relatively thick osseous process renders it difficult to apply a cover-glass. The vestibular surface must be directed upward; it may be recoginzed by the auditory teeth, which are visible when the upper surface is in focus (Fig. 275), while the other portions are not distinct until the tube is depressed and the lower planes are focused. With the low power only the interstices of the auditory teeth are at first visible as dark lines (Fig. 277, labium vestibulare); the papillæ likewise cannot be seen immediately, even with the high power, but become distinct after the second or third day. The chief difficulty lies not in the finishing, but in the proper examination of the object; the picture alters with the slightest change in focus. In Fig. 278, *B*, the membranous spiral lamina is drawn schematically, as seen with the upper surface in focus, therefore only the free surface of the structure, drawn as seen from the side in *A*, is visible. It is clear that in depressing the tube the head-plates of the pillar-cells are no longer visible, but their bodies (as circles in optical section); the reticular membrane likewise disappears and can be seen only when the tube is elevated. The preparation may be stained with picrocarmine and preserved in dilute glycerol. The foregoing directions are intended to apply to the human ear and that of the cat. The labyrinths of children are recommended.

No. 188.—*Sections of the Bony and Membranous Cochlea.*—Remove the cochlea of a child from the labyrinth. The compact osseous substance of the cochlea is surrounded by spongy bone so soft that the latter may be removed with a stout penknife. Having done this, with a chisel make small openings in the cochlea at two or three places, about 1 mm. square, in order to facilitate the penetration of the fixation fluid. Then place the cochlea in 15 c.c. of distilled water plus 5 c.c. of 2 per cent. osmic acid. After twenty-four hours remove the object, wash it for a quarter of an hour in running water, and harden it in about 60 c.c. of gradually-strengthened alcohol. When the hardening is completed decalcify the cochlea in the following mixture: 1 c.c. of a 1 per cent. aqueous solution of palladium chlorid, 10 c.c. of hydrochloric acid, and 100 c.c. of distilled water. Place the cochlea in 100 c.c. of this mixture, which must be frequently changed. When the decalcification is completed the object should be again hardened, embedded in liver, and sectioned. The sections must be made in the long axis of the cochlea. Stain them with picrocarmine; mount in damar. It is not difficult to obtain preparations furnishing a good general view; the vestibular membrane is usually torn, so that the ductus cochlearis and scala vestibuli appear as a common space (Fig. 274). The spiral organ leaves most to

be desired; only very thin sections which pass through the organ vertically furnish intelligible pictures; usually a section contains several inner and outer pillar-cells, in part only fragments of them; the cells of Hensen appear pale and swollen (Fig. 280), so that orientation presents many difficulties to the beginner.

Among animals, the cochlea of the guinea-pig and of the bat are to be recommended; it is not embedded in spongy bone and does not need to be chiseled out and punctured, but can at once be placed in the fixing fluid.

No. 189.—*The Nerves of the Maculæ, Cristæ, and Cochlea.*—For this purpose the ear of the newborn mouse is recommended, treated according to the method given on page 41. The base of the cranium, after removal of the vertex, brain, and lower jaw, is to be placed for from three to four days in the osmio-bichromate mixture and for two days in the silver solution. As a rule it is necessary to employ the double method (p. 42). Cut horizontal and frontal sections through the cranium without decalcifying it. The former are the more readily made.

No. 190.—*The Eustachian Tube.*—To obtain transverse sections (including cartilage and mucosa) the oblique direction of the tube downward, forward, and inward must be ascertained. Cut out the pharyngeal division of the tube together with the surrounding muscles and fix it in 200 or 300 c.c. of Müller's fluid (p. 31). In from three to six weeks wash it in running water and harden it in 100 c.c. of gradually-strengthened alcohol (p. 33). The sections may be stained in Hansen's hematoxylin (p. 36) and mounted in damar (p. 45). For a general view, examine with the low power.

No. 191.—*The Cerumiuous Glands.*—Cut out the ear with the cartilaginous auditory passage close to the bony auditory passage. From the cartilaginous portion cut a piece 1 cm. square and place it in 30 c.c. of absolute alcohol. The tissue may be sectioned on the following day. If it is desired to see the coil and the excretory duct the sections must be tolerably thick (— 0.5 mm.). Nuclear staining with Hansen's hematoxylin (p. 36) may be employed (Fig. 283). Examine thin unstained sections in diluted glycerol; in these the fat-globules and pigment-granules can be seen. The organs of newborn children are especially suitable for this purpose. In adults the tubules are widely dilated and do not furnish good general views. On the other hand, the cuticular border of the gland-cells is distinct in the adult, which in the newborn I miss (compare with Fig. 284).

# XIII.—THE OLFACTORY ORGAN.

In this chapter the entire nasal mucous membrane will be described. The olfactory mucous membrane proper in man is confined to the middle of the superior turbinal bone and to the corresponding portions of the nasal septum; the remaining portions of the nasal fossæ (the accessory nasal spaces included) are covered with respiratory mucous membrane. In addition there is another division in the region of the movable nose (vestibulum nasi) which is clothed by a continuation of the skin. Accordingly three different divisions of the nasal mucous membrane are to be distinguished.

## THE VESTIBULAR REGION.

The mucous membrane of the vestibular region consists of a stratified squamous epithelium and a tunica propria supporting papillæ. Numerous sebaceous glands and the hair-follicles of the stiff nasal hairs (vibrissæ) are embedded in the tunica propria.

## THE RESPIRATORY REGION.

The respiratory portion of the nasal mucous membrane consists of a stratified ciliated columnar epithelium (Fig. 11), that sometimes con-

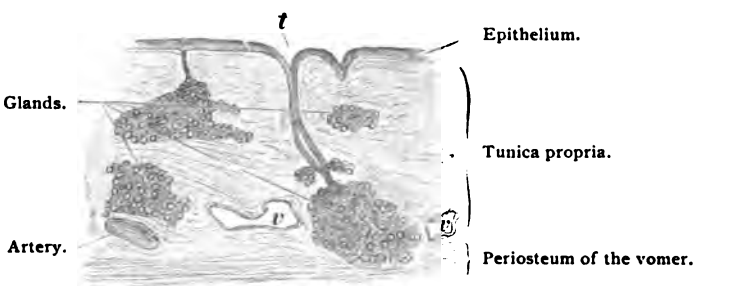


FIG. 285.—THICK VERTICAL SECTION OF RESPIRATORY MUCOUS MEMBRANE OF THE HUMAN NASAL SEPTUM. × 20. The excretory ducts of two glands are visible. *t*. Funnel-shaped depression; *v*, vein. Techn. No. 193.

tains many, sometimes few goblet-cells, and of a conspicuous tunica propria, up to four millimeters thick on the inferior turbinal bone, which

is built of fibrillar connective tissue, of a large, variable number of leucocytes, and toward the epithelial border is condensed to a homogeneous membrana propria provided with minute apertures. These leucocytes are occasionally balled together in solitary nodules and often wander in large numbers through the epithelium into the nasal fossæ (cf. p. 221).

The tunica propria in man contains branched tubular glands, which produce partly mucous and partly serous secretion, therefore are mixed glands. Not infrequently they open in funnel-shaped depressions (Fig. 285, *t*), which are lined by an extension of the superficial epithelium and on the inferior turbinal are perceptible by the unaided eye. In the accessory nasal spaces the epithelium and tunica propria are considerably thinner (— 0.02 mm.), but otherwise of the same structure; the glands are small and few in number.

## THE OLFACTORY REGION.

The mucous membrane of this region by its yellowish-brown color can be macroscopically distinguished from the rosy mucosa of the respiratory division. It consists of an epithelium, the olfactory epithelium, and of a tunica propria. In the olfactory epithelium two forms of cells occur. The one form (Fig. 286, *st*) is cylindrical in its upper half and here contains a yellowish pigment and minute granules, often arranged in longitudinal rows. The lower half is slenderer, the edge is serrated and indented, the inferior end is forked and is said to unite with the similar ends of neighboring cells to form a protoplasmic network. These elements are called *sustentacular cells*. Their usually oval nuclei lie at the same level and in vertical sections occupy a narrow belt, the *zone of the oval nuclei* (Fig. 288). The second form (Fig. 286, *r* and Fig. 287) possesses a spherical nucleus and only in the vicinity of the latter an appreciable amount of protoplasm; from this a slender ciliated cylindrical process extends upward, while from the opposite pole a very delicate process continues directly into the axis-cylinder of a nerve-fiber. These cells, the *olfactory cells*, are ganglion-cells and their lower process a centripetal nerve-fiber. Their round nucleolated nuclei lie at different levels and occupy a broad belt, the *zone of the round nuclei* (Fig. 288, *zr*).

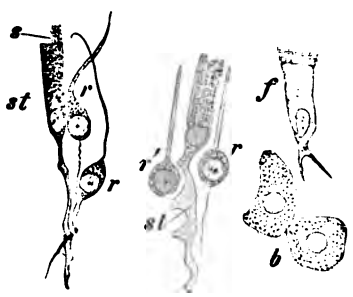


FIG. 286.—ISOLATED CELLS OF THE OLFACTORY MUCOSA OF A RABBIT. × 560. *st*. Sustentacular cells; *s*, extruded mucus resembling cilia; *r*, olfactory cells, at *r'* the lower process has been torn off; *f*, ciliated cells; *b*, cells of olfactory glands. Techn. No. 192.

Occasionally, in the nonnucleated epithelial territory, round nuclei in varying number are found above the zone of the oval nuclei; they either belong to dislocated olfactory cells or are the nuclei of wandering, often pigmented, leucocytes. In addition to these two kinds of cells there are intermediate forms, that sometimes resemble the olfactory elements, sometimes the sustentacular cells. At the border of the epithelium, toward the connective tissue, there is a protoplasmic network furnished with nuclei, the so-called *basal cells* (Fig. 289, *b*). The surface of the epithelium is covered by an extremely delicate homogeneous membrane, the *membrana limitans olfactoria;* it is pierced by the ciliated extremities of the olfactory cells and is itself covered by a peculiar substance, regarded by some authors as a cuticular formation similar to that of the intestinal epithelium, by others as delicate cilia, by still others interpreted as minute particles of discharged mucus (Fig. 286, *s*).

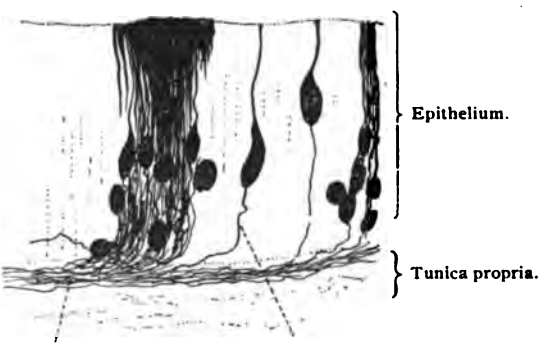


FIG. 287.—VERTICAL SECTION THROUGH THE OLFACTORY REGION OF A YOUNG RAT. × 480. Techn. No. 195.

The *tunica propria* consists of a loose feltwork of rigid connective-tissue fibers intermingled with delicate elastic fibers, which in some animals toward the epithelium (for example, in the cat) is condensed to a structureless membrane. Numerous glands, the so-called *olfactory glands* (Bowman), are embedded in the tunica propria; they are either simple or (for example, in man) branched follicles, in which an excretory duct situated in the epithelium, a body, and a fundus may be distinguished (Fig. 288, *a*). The cells of the body of the glands are pigmented. The olfactory glands (also those of man) until recently were regarded as serous glands, but latterly they have been pronounced mucous glands. The olfactory glands frequently advance beyond the territory of the olfactory mucous membrane and are found in the adjoining portions of the respiratory mucous membrane. The tunica

propria also carries the ramifications of the nerves. The branches of the olfactory nerve are accompanied by processes of the dura and consist

FIG. 288.—VERTICAL SECTION OF THE OLFACTORY MUCOSA OF A RABBIT. × 50. *zo*, Zone of oval, *zr*, zone of round nuclei. *dr*, Olfactory glands; *a*, excretory duct, *k*, body, *g*, fundus. *n*, Branches of olfactory nerve cut transversely; *v*, veins; *ar*, arteries; *b*, bundles of connective tissue in cross-section. Techn. No. 194.

throughout of nonmedullated fibers, that readily separate into their component fibrillæ; the fibers grouped into bundles are the inferior processes of the olfactory cells, which pass in horizontal arches from the epithe-

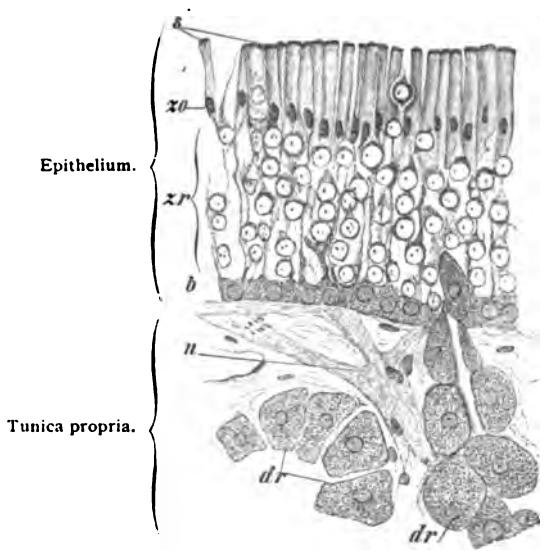


FIG. 289.—VERTICAL SECTION THROUGH THE OLFACTORY MUCOSA OF A RABBIT. × 560. *s*, Cuticular border; *zo*, zone of oval, *zr*, zone of round nuclei; *b*, basal cells; *dr*, portions of olfactory glands, on the right the lower portion of the excretory duct is shown; *n*, branch of the olfactory nerve. Techn. No. 194.

lium and descend into the tunica propria and by union with neighboring bundles form the branches of the olfactory nerve. The terminal ramifications of the fifth nerve lie within the tunica propria; delicate fibers

that ascend to the epithelium and there terminate in free ends possibly belong to the fifth nerve.*

Of the *blood-vessels* of the nasal mucosa the arterial stems run in the deeper strata of the tunica propria (Fig. 285, Fig. 288); they supply a capillary network that reaches close beneath the epithelium. The *veins* are remarkable for their size (Fig. 285) and over the posterior end of the inferior turbinal bone form so dense a network that the tunica propria resembles cavernous tissue.

The *lymph-vessels* form a coarse-meshed net lying in the deeper strata of the tunica propria. The lymph-vessels of the olfactory mucosa may be injected from the subarachnoidal space, through the perineurial sheaths of the branches of the olfactory nerve acquired from the cerebral membranes on passing through the cribriform plate.

Medullated twigs of the fifth nerve may be found in the respiratory as well as in the olfactory mucosa.

## TECHNIC.

No. 192.—*Olfactory Cells.*—Saw open the head of a rabbit in the median line. The olfactory mucosa is easily recognized by its brown color. With fine scissors cut out a small piece of the mucosa, about 5 mm. long, together with the corresponding portion of the turbinal bone, and place it in 20 c.c. of one-third alcohol (p. 20). In five or seven hours transfer the same to 5 c.c. of picrocarmine and on the following day to 10 c.c. of distilled water. In about ten minutes remove the piece and lightly strike it against a slide on which a drop of diluted glycerol has been placed; stirring with the needle is to be avoided. Carefully apply a cover-glass. In addition to many fragments of cells many well-preserved sustentacular elements may be obtained. Very frequently the delicate central process of the olfactory cells is wanting (Fig. 286).

No. 193.—*The Mucous Membrane of the Respiratory Region.*—Cut out a piece about 5 or 10 mm. long from the lower half of the nasal septum; strip off the mucosa and fix and harden it in about 20 c.c. of absolute alcohol (p. 30). Use the nasal mucous membrane of the rabbit's head (No. 192) for thin sections; embed the pieces in liver and stain sections with Hansen's hematoxylin; mount in damar. For general views the mucous membrane of human cadavers answers, which is to be treated in the same manner; thick, unstained sections are to be mounted in diluted glycerol (Fig. 285).

No. 194.—*The Mucous Membrane of the Olfactory Region.*—Remove

* Different authors have described structures in the nasal mucous membrane resembling the taste-buds. It, however, is not certain but that folds of the nasal mucous membrane have been mistaken for these "olfactory-buds."

pieces from 3 to 6 mm. long of the brown mucosa from the upper portion of the nasal septum of a rabbit (No. 192), and place them for three hours in 20 c.c. of Ranvier's alcohol, which somewhat loosens the elements of the olfactory epithelium. Transfer the pieces carefully to 3 c.c. of 2 per cent. osmium solution plus 3 c.c. of distilled water, and place the whole for from fifteen to twenty-four hours in the dark. At the expiration of this time the pieces are to be placed for a half-hour in 20 c.c. of distilled water and then hardened in 30 c.c. of gradually-strengthened alcohol. The hardened pieces are to be embedded in liver and sectioned. Stain the sections from twenty to thirty seconds in Hansen's hematoxylin; mount them in damar.

In order to obtain good views of the *glands* make thick sections transverse to the course of the *nerve-fibers* (Fig. 288). For the exhibition of the *nerve-fibers* and the epithelium thin sections parallel to the course of the fibers are suitable (Fig. 289).

No. 195.—*The nerve-processes of the olfactory cells* may be obtained in preparations made according to Techn. No. 179. In these the duct system of the olfactory glands often is blackened.

## XIV. THE TASTE-BUDS.

The *taste-buds*, the *gustatory organs*, are oval bodies, about 80 $\mu$ long and 40 $\mu$ broad, which are completely embedded in the epithelium of the oral mucous membrane; their base rests upon the tunica propria, the upper end reaches to the surface of the epithelium, which here exhibits a funnel-shaped depression, the *taste-pore*. Each taste-bud consists of two kinds of elongated epithelial-cells; the one is either everywhere of the same diameter or tapers at the basal end, which occasion-

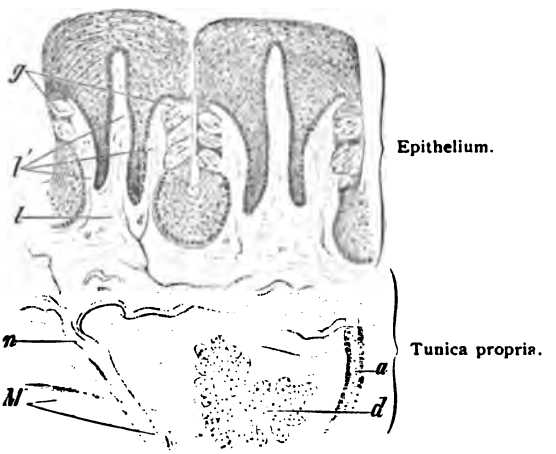


FIG. 290.—VERTICAL SECTION OF TWO RIDGES OF THE PAPILLA FOLIATA OF A RABBIT. × 80. Each ridge, *l*, bears secondary ridges, *l'*; *g*, taste-buds; *n*, medullated nerves; *d*, serous gland; *a*, portion of an excretory duct of a serous gland; *M*, muscle-fibers of the tongue. Techn. No. 197.

ally is forked, while the upper end is prolonged to a fine point; the protoplasm is clear. These cells constitute the bulk of the taste-bud, are principally situated at the periphery of the bud, and are called *tegmental cells*. They serve as support and sheath for the *gustatory cells*, which are the real percipient epithelial elements. The gustatory cells are small and only slightly thickened where the nucleus is situated; the latter is sometimes nearer the lower end, sometimes in the middle, rarely at the upper end of the cell. The upper division of the cell is cylindrical, or more frequently conical, and bears on its free end a stiff,

refractile, hair-like process, a cuticular formation (Fig. 291); the lower division is sometimes slender, sometimes thick, and terminates in a blunted end or in a triangular foot, without however extending into

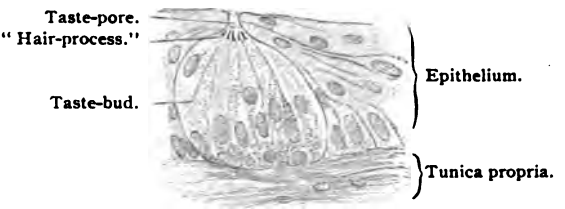


FIG. 291.—FROM A VERTICAL SECTION OF THE PAPILLA FOLIATA OF A RABBIT. × 560. Techn. No. 197.

the connective tissue of the mucosa. Their protoplasm is granular. Not infrequently many leucocytes are found in the interior of the taste-bud.

The taste-buds chiefly occur in the lateral walls of the circumvallate

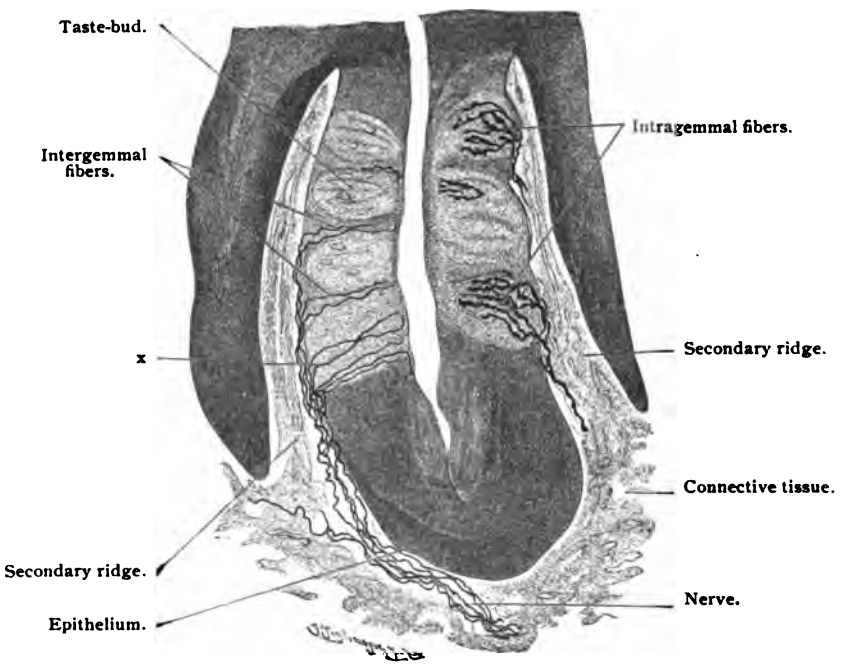


FIG. 292.—FROM A VERTICAL SECTION OF THE FOLIATE PAPILLA OF A RABBIT. × 220. At x the intergemmal fibers lie upon a taste-bud. For orientation compare with Fig. 290. Techn. No. 199.

papillæ and on the ridges of the papillæ foliatæ, in smaller number on the fungiform papillæ, on the soft palate, and on the posterior surface of the epiglottis.

The conjecture that the terminal ramifications of the glossopharyngeal nerve have the same anatomic relation to the gustatory cells that the olfactory nerve-fibers have to the olfactory cells has been shown to be erroneous. The terminal branches of the glossopharyngeal nerve consist of medullated and gray nerve-fibers beset with microscopic (sympathetic) ganglia,* which form a dense plexus in the tunica propria, from which numerous branches arise. Some of the latter perhaps terminate in the connective tissue in end-bulbs, but the majority of the gray fibers penetrate into the epithelium. Here two kinds of fibers may be distinguished. The one kind, the "intragemmal" fibers, enter the taste-buds, divide, and form a plexus beset with numerous conspicuous varicosities that extends up to the taste-pore; these fibers do not anastomose with one another, nor do they connect with the gustatory cells, but all terminate in free ends. The other, the smoother "intergemmal" fibers, penetrate the epithelial areas between the taste-buds and, usually without dividing, extend to the uppermost strata of the epithelium.

## TECHNIC.

No. 196.—*For orientation with regard to the number and position of the taste-buds* proceed according to the method in No. 96. Suitable objects are the circumvallate papillæ of any animal and the papillæ foliatæ of the rabbit. The latter consist of elevated groups of parallel folds of the mucosa, found one on either edge of the root of the tongue. In moderately thin sections vertical to the long axis of the folds, with the low power, the taste-buds may be recognized as clear spots.

No. 197.—*The Structure of the Taste-buds.*—Dissect off with scissors a papilla foliata of a rabbit, with as little as possible of the subjacent muscle substance. Pin the piece with spines on a cork-stopper, the muscle side toward the cork, and expose it for one hour to the vapor of osmic acid (see further p. 32, 7). Thin sections of the hardened preparation embedded in liver are to be stained thirty seconds in Hansen's hematoxylin and mounted in damar (Fig. 290).

No. 198.—*Exhibition of the Nerves.*—With scissors cut out a circumvallate papilla (without the wall), and place it for ten minutes in the filtered juice of a lemon; then transfer it to 5 c.c. of a 1 per cent. gold-chlorid solution and place the whole for one hour in the dark. Lift the papilla with wooden rods from the gold-chlorid solution into a watch-glass with distilled water and wash it by moving it to and fro. Transfer it to 20 c.c. of distilled water to which three drops of acetic acid have been added. In this expose the papilla to daylight until the reduction is

* Whether the so-called "taste-granules" beneath the epithelium of the papillæ foliatæ are multipolar nerve-cells is very questionable; a nerve-process has not as yet been demonstrated.

completed, which usually requires three days. Harden the papilla in the dark in 30 c.c. of gradually-strengthened alcohol. Embed the object and make the thinnest possible sections. Mount in damar. The nerve-fibers are dark-red to black; the gustatory cells are also dark (compare with Fig. 292). The foliate papillæ of the rabbit are not suitable for this method.

No. 199.—Place the papillæ foliatæ of a rabbit for three days in the osmio-bichromate mixture, for two days in the silver solution (p. 41). The double method is recommended. The intergemmal fibers are more numerous and more readily blackened than the intragemmal fibers, which are exceedingly delicate (Fig. 292). Frequently single cortical and gustatory cells become blackened.

# APPENDIX.

## MICROTOME TECHNIC.

### THE MICROTOME.

The most useful microtomes are constructed according to two different principles.

The principle of the one kind consists therein, that the object to be sectioned is elevated by the shifting of the object-holder up an inclined plane.

In the other form, the object is elevated in a vertical direction by a micrometer-screw.

Both kinds are excellent instruments.*

All parts of the microtome should be kept as clean as possible. When not in use it should be protected from dust by covering it with a light wooden case. The slideway in which the knife moves must be kept scrupulously clean. It should be occasionally cleansed with a cloth moistened in benzin and should then be freely lubricated with vaselin, so that the sliding-block will pass evenly throughout the entire slideway at the lightest touch. Especial care must be bestowed upon the knife. Only with a very sharp knife can very thin sections be made or ribbon-cutting be done. A really sharp knife should easily pass through a thin hair held at one end between the fingers.

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* The workmanship of the sliding microtomes of Thoma, made by Jung in Heidelberg, is exquisite, as I know from my own experience. The size No. IV is especially to be recommended. For several years I have used the microtome of Schanze in Leipzig, Model B, No. 9, the construction of which leaves nothing further to be desired. The microtomes constructed on the same principle, by G. Mihe in Hildesheim, are also to be highly recommended, and very good are those of A. Becker in Göttingen.

*Editor's remark:* The *automatic microtome of Minot* is widely used, particularly in American laboratories. This instrument is distinguished from those above described by the great rapidity with which it can be worked. Therefore it is to be highly recommended, especially for the preparation of long series of paraffin-sections attached one to the other in the form of a ribbon ("ribbon-cutting"). In exactness of action it is hardly surpassed by the German models, from which it altogether differs in construction. The object is moved by the rotation of a wheel in a vertical direction up and down across the edge of a knife and previous to every cut is advanced toward the knife a certain distance, which is regulated by an automatic micrometer-screw.

It is difficult to recommend in particular any one of the microtomes mentioned. Each has its advantages and disadvantages, and to be successfully used demands a certain amount of experience and practice, which determines the individual preference for a certain instrument.

The Minot-microtome is made by E. Zimmermann, Leipzig, Germany, and in the United States by the Bausch & Lomb Optical Co., New York and Rochester, N. Y. The latter also make a very satisfactory sliding microtome, on the principle of the Schanze-microtome.

## EMBEDDING.

### The Paraffin Method.

The following materials and apparatus are required :—

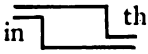
1. *Paraffin :* two kinds, a soft (melting point 45° Celsius) and a hard (melting point 52° Celsius). Of this prepare a mixture which melts at 50° Celsius. On the proper proportions of the two sorts of paraffin in the mixture much depends ; many a failure is due to an unsatisfactory mixture. The precise proportions cannot be given because the consistence of the paraffin depends in a great measure on the outer temperature. Then, too, hard objects, as well as the cutting of very thin sections, require a harder mixture than usual. For winter, at a room-temperature of 20° Celsius, a mixture of 30 grams of soft and 25 grams of hard paraffin* answers for most purposes.

2. *Chloroform :* 20 c.c.

3. *Paraffin-chloroform :* a saturated solution (5 grams of the paraffin mixture and 25 c.c. of chloroform). This solution is liquid at room-temperature.

4. *An embedding oven* of block-tin with double walls, between which is a space to be filled with water.† A small gas-burner is to be placed beneath the oven. On top there are three openings ; two lead into the space between the walls, into one a Reichert thermo-regulator ‡ is to be inserted, into the other a thermometer ; the third opening leads into the air-space or oven and into this a second thermometer is to be inserted. The front wall consists of a glass plate which slides up and down in grooves. The interior of the oven is divided into three compartments by means of two adjustable shelves. The oven should be 25 cm. long, 15 cm. high, and 15 cm. deep. The embedding oven with its accessories is indispensable if much embedding in paraffin is to be done ; but the paraffin may be melted on a water-bath and kept liquid with a small spirit-flame.

5. *An Embedding Frame.*—This consists of two adjustable bent metal frames, placed together




Instead of this frame little paper trays made of stiff paper or cardboard can be used.

The objects to be embedded must be absolutely free from water and to this end should have lain three days in absolute alcohol which has been changed several times ; they are then to be transferred to a bottle containing 20 c.c. of chloroform, in which they should remain

* To be obtained of Dr. Grübler, of Leipzig.

† Made by R. Jung, Heidelberg, Germany, and in the United States by the Bausch & Lomb Optical Co., New York.

‡ To be obtained of the Bausch & Lomb Optical Co., New York.

until the following day. From this the objects should be carried to the solution of paraffin in chloroform and in from two to eight hours, according to their size, transferred to a capsule containing melted (but not too hot) paraffin. In about a half hour the objects are to be transferred to a second capsule with melted paraffin,* where, according to their size, they are to remain from one to five hours.† The paraffin should not be heated more than two or three degrees above its melting point; for the mixture advised the air in the oven should have a temperature of 50° Celsius.

When the objects have been in the paraffin bath the required length of time, place a slide in a broad dish and on this the embedding frame, into which paraffin and object now are to be poured. Then, while the paraffin is still fluid, with a heated needle place the object in the desired position; so soon as this is done carefully pour cold water into the dish until it reaches the upper margin of the frame; the paraffin will at once begin to harden, whereupon more water may be added until the entire frame is submerged. By this manipulation the paraffin hardens into a homogeneous mass, whereas otherwise it is apt to crystallize and is then difficult to cut and also has an injurious influence on the structure of the embedded tissues. In about ten minutes the metal frames may be removed; the paraffin block should be allowed to remain in the water on the slide until it is completely hard.

The embedded object may be sectioned in a half hour. In case it is to be used later mark it with a needle. In the paraffin the object can be kept for an indefinite period.

### The Celloidin Method.

Two solutions are required :—

*a.* A thin solution of about 30 grams of celloidin cut into cubes dissolved in 60 c.c. of a mixture of equal parts of absolute alcohol and ether.

*b.* A somewhat thicker solution of 30 grams of celloidin dissolved in 40 c.c. of a mixture of equal parts of absolute alcohol and ether. This solution has the consistence of a thick syrup.

Both solutions should be kept in wide-mouthed bottles. If they become too thick they may be thinned by the addition of some of the alcohol-ether mixture. After a time the solutions become turbid and milky; it is better then to let them dry completely and to redissolve the pieces in the alcohol-ether mixture.

The tissues to be embedded must be completely free from water and must have lain one or two days in absolute alcohol which has been changed several times. From this the objects should be transferred to the thin and on the following day to the thick celloidin solution. In the latter, the objects may remain for an indefinite length of time. Usually

* If the paraffin has been melted on a water-bath, place the flame at such a distance that the surface of the paraffin remains covered by a thin film.

† This is sufficient for all cases; for small objects from one to two hours will be enough.

they are sufficiently permeated after twenty-four hours, but large objects enclosing many spaces must remain in the thick solution about eight days. The object should then be quickly placed on a cork-stopper and some celloidin poured over it. In doing this care must be taken not to press the object against the cork, lest it become detached. There should be a stratum of celloidin one or two millimeters thick between the cork and the object.* Now the whole is to be placed under a bell-glass to slowly dry; the bell-glass should not be air-tight, and to avoid this should be supported on one side on a needle or something similar. Delicate objects dry in a half hour, larger objects in four hours; they are then to be placed in a glass jar with 30 c.c. of 80 per cent. alcohol. In order that the objects may be submerged, glue the under surface of the cork-stopper by means of celloidin to the inner surface of the lid of the jar. On the following day the alcohol should be replaced by 70 per cent. alcohol, in which the tissue may remain an indefinite length of time.

In order to cut thin sections the celloidin must be hardened; for this purpose transfer the objects embedded in celloidin from the 80 per cent. alcohol for two days or longer into an alcohol-glycerol mixture (80 per cent. alcohol one part, pure concentrated glycerol from six to ten parts). The larger the proportion of glycerol to alcohol, the harder the celloidin becomes. This mixture may be differently prepared; an extreme limit is one part of alcohol to 30 parts of glycerol. Still greater difference in the proportions produces strong curling of the sections. In order to prevent the yielding of the elastic celloidin block, dry it carefully with filter-paper when it is removed from the alcohol-glycerol mixture, make a pair of lateral incisions and dip it into liquid paraffin; such blocks cannot be preserved dry, they must be returned to the alcohol-glycerol mixture.

Preparations fixed by Golgi's method require special treatment, since the absolute alcohol has an injurious influence if the object remains in it beyond one hour. When the tissue is taken from the silver solution it is to be placed in 30 c.c. of 95 per cent. alcohol, fifteen or twenty minutes, then hardened in absolute alcohol for fifteen minutes, then placed in the thin celloidin solution for five minutes. Meanwhile, in the previously smoothed lateral surface of a broad piece of elder-pith make an excavation just large enough to take in the whole preparation; insert it, cover it with celloidin solution, fit a second piece of elder-pith on the first, pour on more celloidin, and place the whole for five minutes under a bell-glass to dry; then transfer it to 80 per cent. alcohol for five minutes, and cut sections with a knife flooded with 80 per cent. alcohol. The microtome is altogether unnecessary; satisfactory sections can easily be cut free-hand. If the microtome be used, the thickness of the sections should vary from 40 to 120 $\mu$. The elder-pith should be trimmed off so that only a small border (1 mm.) surrounds the celloidin.

* This stratum must not be thicker; even well-hardened celloidin is elastic, and a thick layer would cause the object to give in sectioning.

# SECTIONING.

## Paraffin Objects.

*With the Knife Placed Obliquely.*—The paraffin block containing the tissue is to be secured in a hollow cylinder coated with hard paraffin (in the Thoma microtome) or (in the microtome of Schanze) to a little plate adjoining the clamp. With the latter the plate is simply warmed and the paraffin block glued to it by pressure. In the case of the cylinder, warm it and also the base of the paraffin block; press the latter lightly into the cylinder and by means of a heated needle inserted between them establish a firm union. In order quickly to cool the paraffin place the cylinder or the plate for five minutes in cold water. The projecting portion of the paraffin block containing the object should then be trimmed to a four-sided column, the base of which is a right-angled parallelogram.

The column must not be taller than one centimeter, and the object should be covered by a layer of paraffin not over one or two millimeters thick. The cylinder (or the plate) with the object should now be placed in the microtome. Sections are to be cut with the blade of the knife dry. The position of the knife depends on the nature of the object.

*Sectioning with the Knife Placed Obliquely.*—If the object is large and of unequal resistance the knife should be so clamped that it forms a very acute angle with the long axis of the microtome. The paraffin block should so stand that the knife strikes it first on one corner. The knife should be moved slowly in the slideway and pressure upon it should be carefully avoided.

*Sectioning with the Knife Placed Transversely.*—Screw the knife down perpendicular to the long axis of the microtome, turn the paraffin block so that the blade will strike it first on a flat surface. The knife should be rapidly moved with a planing movement and then the sections will adhere to one another at their edges and form long ribbons. When the paraffin is of the right consistence the first section lies smoothly on the blade and is shoved by the second section in the direction of the back of the blade. If however the first sections show an inclination to curl and fall over the edge, they must then be carefully held with a delicate sable brush and led back to the right position. Ribbon-cutting is most successful when the sections have a thickness of 0.01 of a millimeter; thicker sections easily curl and do not readily adhere to one another at their edges.

## Obstacles in Sectioning and their Remedy.

Every one who has worked with paraffin is probably able to explain many an unsuccessful attempt.

1. The knife glides over the object and cuts a partial section or none. The reason for this may lie in the microtome; the slideway may not be clean; examine the vertical portion of the slideway. Or the knife is not sharp enough, or the under surface has paraffin attached to

it; in the latter case remove the knife and with a cloth wetted with turpentine carefully cleanse it. Knives with thin backs buckle if the distal end of the blade is used; thus it happens that when the knife is obliquely placed the blade cuts the tissue only at the edge where it first touches and glides over the rest without cutting it. In microtomes of earlier construction the cause of this often lies in the unsatisfactory manner in which the block of paraffin is secured.

Secondly, the trouble may be found in the object; it may be too hard, or of very unequal resistance, or poorly embedded; in the latter case there are two possibilities. Either the preparation was not thoroughly dehydrated, in which case it exhibits opaque spots or it still contains chloroform; in this case it is soft, and light pressure with a needle on the surface leaves a mark or even presses out fluid. In both cases the procedure of embedding must be repeated, reversing the series of processes to the absolute alcohol (in the latter case to the paraffin bath).

Finally, the consistence of the paraffin may be at fault.

2. The sections curl. This can be prevented by holding a small sable brush or bent needle lightly against the sections as they are cut.* The cause of this curling lies in the hardness of the paraffin, which is also responsible for—

3. The sections break. The usefulness of the paraffin depends in a high degree on the outer temperature. If the paraffin is too hard do not endeavor to reduce its consistence by the admixture of soft paraffin, —this is the last resource,—but employ simpler measures. Cut the sections near a stove or near a lamp; often slight warming of the knife is sufficient. Even very good paraffin crumbles when cut with a cold knife.

4. The sections fold and become pressed together. As a result of this the sectioned objects acquire a false form. The reason for this lies in a too soft paraffin. This difficulty may be overcome by frequently placing the block in cold water or by cutting the sections in a cold room (in summer, in the morning hours).

### Celloidin Objects.

The embedded object is to be trimmed so that it is surrounded by a stratum of celloidin only one or two millimeters thick; clamp the knife obliquely, so that it makes a very acute angle with the long axis of the microtome. The knife must be moistened with 70 per cent. alcohol by means of a sable brush; this must be done after every second or third section is cut. The sections should be removed with a sable brush and transferred to a dish containing 70 per cent. alcohol. Very thin sections (less than 0.02 mm.) cannot be cut unless the celloidin has been hardened.

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* A "section-smoother" for microtomes in which the object is elevated vertically is made by Kleinert of Breslau. See further, Born, "Zeitschr. f. wissensch. Mikroskopie," Bd. x, p. 157.

## PRESERVATION OF SECTIONS.

### Paraffin Objects.

If the sections are not very thin and are not in ribbons, they may be placed in a capsule with 5 c.c. of turpentine and when the paraffin is dissolved transferred to a second capsule with turpentine. From this the sections, if the tissue has been stained in bulk, are carried to a slide and mounted according to the directions given on page 44. If the sections are unstained, transfer them from turpentine to 5 c.c. of ninety-five per cent. alcohol, which is to be changed in five minutes. In another two minutes the sections may be stained. In the case of serial sections and very thin sections, it is necessary to fasten the dry sections on the slide. The slide must be absolutely clean; wash it with alcohol and dry it with a clean, *not oily*, cloth or place it for a half hour in cold soap-suds. On the well-dried slide arrange the sections (or portion of the "ribbon"), and at the edge of the same place a drop of distilled water by means of a delicate sable brush. Another section (or portion of the ribbon) is now placed on the slide, another drop of water added, and so on until the slide is covered. It does not matter if the sections float. Pass the slide through a spirit-flame or place it for from one to three minutes in the oven;* on being slightly warmed, the sections spread out flat and smooth. Then arrange them with a needle and by slightly inclining the slide let the water flow off, or absorb it with a strip of filter-paper and, protected from dust, let the whole dry. On the following day pour turpentine over the slide and if the sections are already stained mount them in damar. In case the sections are not stained the turpentine is to be wiped off and the slide placed in ninety-five per cent. alcohol.† After five minutes take the slide from the alcohol, which is to be quickly wiped off around the sections, breathed upon, and either placed in the stain or covered with a drop of the solution. Then slowly transfer the slide to a dish with distilled water and preserve it in dilute glycerol (p. 45), or with the customary preliminary treatment with ninety-five per cent. alcohol and oil of bergamot (p. 45), mount it in damar.

### Celloidin Objects.

Place the sections in a dish containing 20 c.c. of ninety per cent. alcohol. If the tissue has not been previously stained in bulk, staining in bulk to be preferred, the sections may be subsequently stained; but

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* The paraffin must not be allowed to melt; the resulting mixture of melted paraffin and water is not soluble in turpentine.

† The turpentine, also the alcohol, must be quickly wiped off, because the sections are rendered useless if they are allowed to become dry. Care must also be exercised in placing the staining fluid on the sections, which it should completely cover. Loosening of the sections occurs when there is not enough water between the section and the slide—the water must be evenly diffused between the two. The section may also be fastened to the cover-glass, but this method necessitates the use of larger quantities of the staining solution, alcohol, and other reagents.

anilin colors cannot be used, since they also stain the celloidin ; even hematoxylin imparts a light-blue tint to the celloidin. The sections must not be placed in stronger alcohol, since this dissolves the celloidin ; they are to be taken from the ninety per cent. alcohol and placed in chemically pure amyl alcohol and then transferred to xylol ; when the clearing is completed mount them in xylol-balsam.

Serial sections of celloidin objects are used only for special purposes, for example, for the central nervous system. See the articles by Wiegert in the "Zeitschrift für wissenschaftliche Mikroskopie," Bd. ii., p. 490, Bd. iii., p. 480, Bd. iv., p. 209. The negative varnish recommended in the article is to be obtained of Dr. Grübler.

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